No. 200200029

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Paragon Seed, Inc.

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSE , OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSES, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

LETTUCE

'Queen of Hearts'

In Testimony Whereof, I have hereunto set my hand and caused the seal of the Plant Variety Protection Office to be affixed at the City of Washington, D.C. this nineteenth day of September, in the year two thousand and five.

Attest:

Commissioner
Plant Variety Protection Office
Agricultural Marketing Service

of Agriculture

REPRODUCE LOCALLY. *Include form number and date on all reproductions.* FORM APPROVED - OMB NO. 0581-0055

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE DIVISION - PLANT VARIETY PROTECTION OFFICE

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE

(Instructions and information collection burden statement on reverse)

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a).

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF APPLICANT(S) *(as it is to appear on the Certificate)*
Paragon Seed, Inc.

2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER
PP 125

3. VARIETY NAME
Queen of Hearts

4. ADDRESS *(Street and No., or R.F.D. No., City, State, and ZIP Code, and Country)*
P.O. Box 1906
Salinas, California 93902

5. TELEPHONE *(include area code)*
831-753-2100

6. FAX *(include area code)*
831-753-1470

FOR OFFICIAL USE ONLY

FILING DATE
PVPO NUMBER: 200200029
DATE: November 26, 2001

FEES RECEIVED
FILING AND EXAMINATION FEE: $ 2705 -
DATE: November 26, 2001
CERTIFICATION FEE: $ 682.00
DATE: August 2, 2005

7. GENUS AND SPECIES NAME
Lactuca sativa L.

8. FAMILY NAME *(Botanical)*
Compositae

9. CROP KIND NAME *(Common name)*
Lettuce romaine type

10. IF THE APPLICANT NAMED IS NOT A "PERSON", GIVE FORM OF ORGANIZATION *(corporation, partnership, association, etc.) (Common name)*
Corporation

11. IF INCORPORATED, GIVE STATE OF INCORPORATION
California

12. DATE OF INCORPORATION
March 7, 1994

13. NAME AND ADDRESS OF APPLICANT REPRESENTATIVE(S), IF ANY, TO SERVE IN THIS APPLICATION AND RECEIVE ALL PAPERS
Victor Heintzberger
Paragon Seed, Inc.
P.O. Box 1906
Salinas, California 93902-1906

14. TELEPHONE *(include area code)*
831-753-2100

15. FAX *(include area code)*
831-753-1470

16. CHECK APPROPRIATE BOX FOR EACH ATTACHMENT SUBMITTED *(Follow instructions on reverse)*

a. [X] Exhibit A. Origin and Breeding History of the Variety
b. [X] Exhibit B. Statement of Distinctness
c. [X] Exhibit C. Objective Description of the Variety
d. [ ] Exhibit D. Additional Description of the Variety
e. [X] Exhibit E. Statement of the Basis of the Applicant's Ownership
f. [X] Voucher Sample *(2,500 viable untreated seeds or, for tuber propagated varieties verification that tissue culture will be deposited and maintained in a public repository)*
g. [X] Filing and Examination Fee ($2,460), made payable to "Treasurer of the United States" *(Mail to PVPO)*

17. DOES THE APPLICANT SPECIFY THAT SEED OF THIS VARIETY BE SOLD BY VARIETY NAME ONLY, AS A CLASS OF CERTIFIED SEED? *(See Section 83(a) of the Plant Variety Protection Act)?*
[ ] YES *(If "yes," answer items 18 and 19 below)* [X] NO *(If "no," go to item 20)*

18. DOES THE APPLICANT SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF GENERATIONS?
[ ] YES [ ] NO

19. IF "YES" TO ITEM 18, WHICH CLASSES OF PRODUCTION BEYOND BREEDER SEED?
[ ] FOUNDATION [ ] REGISTERED [ ] CERTIFIED

20. HAS THE VARIETY OR A HYBRID PRODUCED FROM THE VARIETY BEEN RELEASED, USED, OFFERED FOR SALE, OR MARKETED IN THE U.S. OR OTHER COUNTRIES?
[X] YES *(If "yes," give names of countries and dates)* [ ] NO

California USA March 02,2001

21. The applicant(s) declare that a viable sample of basic seed of the variety will be furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate.

The undersigned applicant(s) is(are) the owner(s) of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 41, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

Applicant(s) is(are) informed that false representation herein can jeopardize protection and result in penalties.

SIGNATURE OF APPLICANT *(Owner(s))*		SIGNATURE OF APPLICANT *(Owner(s))*	
NAME *(Please print or type)* Victor Heintzberger		NAME *(Please print or type)*	
CAPACITY OR TITLE President	DATE 11/19/01	CAPACITY OR TITLE	DATE

REPRODUCE LOCALLY. *Include form number and date on all reproductions*

Form Approved - OMB No. 0581-0055

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE AND TECHNOLOGY - PLANT VARIETY PROTECTION OFFICE

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE

(Instructions and information collection burden statement on reverse)

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF OWNER
PARAGON SEED, INC.

2. TEMPORARY DESIGNATION OR EXPERIMENTAL NAME
PP 125

3. VARIETY NAME
QUEEN OF HEARTS

4. ADDRESS *(Street and No., or R.F.D. No., City, State, and ZIP Code, and Country)*
P.O. BOX 1906
SALINAS, CALIFORNIA 93902-1906

5. TELEPHONE *(include area code)*
831-753-2100

6. FAX *(include area code)*
831-753-1470

FOR OFFICIAL USE ONLY

PVPO NUMBER

FILING DATE
20020029

7. IF THE OWNER NAMED IS NOT A "PERSON", GIVE FORM OF ORGANIZATION *(corporation, partnership, association, etc.)*
CORPORATION

8. IF INCORPORATED, GIVE STATE OF INCORPORATION
CALIFORNIA

9. DATE OF INCORPORATION
MARCH 7, 1994

10. NAME AND ADDRESS OF OWNER REPRESENTATIVE(S) TO SERVE IN THIS APPLICATION. *(First person listed will receive all papers)*
VICTOR HEINTZBERGER
PARAGON SEED, INC.
P.O. BOX 1906
SALINAS, CALIFORNIA 93902-1906

FEES RECEIVED

FILING AND EXAMINATION FEES:
$
DATE

CERTIFICATION FEE:
$
DATE

11. TELEPHONE *(Include area code)*
831-753-2100

12. FAX *(Include area code)*
831-753-1470

13. E-MAIL
LETTUCESEED@aol.com

14. CROP KIND *(Common Name)*
LETTUCE

15. GENUS AND SPECIES NAME OF CROP
Lactuca sativa L.

16. FAMILY NAME *(Botanical)*
COMPOSITAE

17. IS THE VARIETY A FIRST GENERATION HYBRID?
☐ YES ☒ NO

18. CHECK APPROPRIATE BOX FOR EACH ATTACHMENT SUBMITTED (Follow instructions on reverse)

a. ☒ Exhibit A. Origin and Breeding History of the Variety
b. ☒ Exhibit B. Statement of Distinctness
c. ☒ Exhibit C. Objective Description of Variety
d. ☐ Exhibit D. Additional Description of the Variety *(Optional)*
e. ☒ Exhibit E. Statement of the Basis of the Owner's Ownership
f. ☒ Voucher Sample *(2,500 viable untreated seeds or, for tuber propagated varieties, verification that tissue culture will be deposited and maintained in an approved public repository)*
g. ☒ Filing and Examination Fee ($2,705), made payable to "Treasurer of the United States" *(Mail to the Plant Variety Protection Office)*

19. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE SOLD AS A CLASS OF CERTIFIED SEED? *See Section 83(a) of the Plant Variety Protection Act)*
☐ YES *(If "yes", answer items 20 and 21 below)* ☒ NO *(If "no", go to item 22)*

20. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF CLASSES? ☐ YES ☐ NO
IF YES, WHICH CLASSES? ☐ FOUNDATION ☐ REGISTERED ☐ CERTIFIED

21. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF GENERATIONS? ☐ YES ☐ NO
IF YES, SPECIFY THE NUMBER 1,2,3, *etc.* ☐ FOUNDATION ☐ REGISTERED ☐ CERTIFIED
(If additional explanation is necessary, please use the space indicated on the reverse.)

22. HAS THE VARIETY (INCLUDING ANY HARVESTED MATERIAL) OR A HYBRID PRODUCED FROM THIS VARIETY BEEN SOLD, DISPOSED OF, TRANSFERRED, OR USED IN THE U. S. OR OTHER COUNTRIES?
☒ YES ☐ NO
IF YES, YOU MUST PROVIDE THE DATE OF FIRST SALE, DISPOSITION, TRANSFER, OR USE FOR EACH COUNTRY AND THE CIRCUMSTANCES. *(Please use space indicated on reverse.)*

23. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT *(PLANT BREEDER'S RIGHT OR PATENT)*?
☐ YES ☒ NO
IF YES, PLEASE GIVE COUNTRY, DATE OF FILING OR ISSUANCE AND ASSIGNED REFERENCE NUMBER. *(Please use space indicated on reverse.)*

24. The owners declare that a viable sample of basic seed of the variety will be furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate.

The undersigned owner(s) is(are) the owner of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 42, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

Owner(s) is(are) informed that false representation herein can jeopardize protection and result in penalties.

SIGNATURE OF OWNER
Victor Heintzberger

NAME *(Please print or type)*
VICTOR HEINTZBERGER

CAPACITY OR TITLE
2 PRESIDENT

DATE
12/10/01

SIGNATURE OF OWNER

NAME *(Please print or type)*

CAPACITY OR TITLE

DATE

GENERAL: To be effectively filed with the Plant Variety Protection Office (PVPO), **ALL** of the following items must be received in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E; (3) for a seed reproduced variety at least 2,500 viable untreated seeds, for a hybrid variety at least 2,500 untreated seeds of each line necessary to reproduce the variety, or for tuber reproduced varieties verification that a viable *(in the sense that it will reproduce an entire plant)* tissue culture will be deposited and maintained in an approved public repository; (4) check drawn on a U.S. bank for $2,70 ($320 filing fee and $2,385 examination fee), payable to "Treasurer of the United States" *(See Section 97.6 of the Regulations and Rules of Practice.)* Partial applications will be held in the PVPO for not more than 90 days, then returned to the applicant as unfiled. Mail application and other requirements to Plant Varie Protection Office, AMS, USDA, Room 500, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. **DO NOT** use masking materials to make corrections. If a certificate is allowed, you will be requested to send a check payable to "Treasurer of the United States" in the amou of $320 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

20020029

Plant Variety Protection Office
Telephone: (301) 504-5518
FAX: (301) 504-5291
Homepage: http://www.ams.usda.gov/science/pvpo/pvp.htm

ITEM

18a. Give:
(1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;
(2) the details of subsequent stages of selection and multiplication;
(3) evidence of uniformity and stability; and
(4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified

18b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:

(1) identify these varieties and state all differences objectively;
(2) attach statistical data for characters expressed numerically and demonstrate that these are clear differences; and
(3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.

18c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.

18d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, *etc.*

18e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.

19. If "Yes" is specified *(seed of this variety be sold by variety name only, as a class of certified seed)*, the applicant **MAY NOT** reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. *(See Regulations and Rules of Practice, Section 97.103).*

22. *See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.*

23. *See Section 55 of the Act for instructions on claiming the benefit of an earlier filing date.*

21. **CONTINUED FROM FRONT** *(Please provide a statement as to the limitation and sequence of generations that may be certified.)*

22. **CONTINUED FROM FRONT** *(Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)*

FIRST COMMERCIAL SALE 3/02/01 CALIFORNIA, U.S.A
TRIAL SAMPLE TO ELSOMS SEED PTY SPALDING ENGLAND 5/29/01

23. **CONTINUED FROM FRONT** *(Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)*

NOTES: It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. There is no charge for filing a change of address. The fee for filing a change of ownership or assignment or any modification of owner's name is specified in Section 97.175 of the regulations. *(See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)*

To avoid conflict with other variety names in use, the applicant must check the variety names proposed by contacting: Seed Branch, AMS, USDA, Room 213, Building 306, Beltsville Agricultural Research Center–East, Beltsville, MD 20705. Telephone: (301) 504-8089. http://www.ams.usda.gov/lsg/seed/ls-sd.htm

STD-470 (04-01) designed by the Plant Variety Protection Office with WordPerfect 6.0a. Replaces STD-470 (02-99) which is obsolete.

EXHIBIT A

BREEDING HISTORY QUEEN OF HEARTS

The objective of this breeding project was to develop a tall, slightly cupping romaine type lettuce that would fit the needs of a new market of food service products. The growing market of "hearts" romaine requires a tall, cylindrical plant type with a slightly cupping apex with a bright yellow interior color. This plant when cut and trimmed will produce a product with an approximately fifteen to twenty leaf count and an average weight of 100 grams. It is important that the core length be in the range of four to six centimeters.

In the Salinas Valley of California, new introductions are required to have some level of Corky Root Rot resistance to gain grower acceptance. Tipburn resistance is also important, as well as weight, bolt tolerance, and mildew resistance.

With this in mind, Paragon PIC (Parris Island Cos) was chosen as the mother plant. Paragon PIC is a large to very large romaine lettuce with excellent weight, moderate girth, large core diameter, and excellent uniformity of type. Paragon PIC has also shown excellent tipburn tolerance in five years of commercial production. Color of Paragon PIC is similar to Parris Island Cos, a medium vanguard green. Seed color of Paragon PIC is white.

Presidio was chosen as the pollen parent for its dark green savoyed leaf, yellow internal color, and Corky Root Rot resistance. Presideo is dark green in color with a shiny leaf reflectance. Presidio is susceptible to tipburn. Seed color of Presidio is black.

The cross between Paragon PIC x Presidio was made on August 04, 1996. Paragon Seed, Inc. personnel made this cross near Corcoran, California.

F1 Seed color was white (silver)

In the fall of 1996, 21 f1 seeds were germinated in petrie dishes and later transplanted into gallon pots in a greenhouse near the Salinas Valley. The plants were designated PP1 through PP21. These plants were grown under lights through the winter and seed harvested in the spring of 1997.
The following plants were harvested from the greenhouse:

PP1	white seed	PP15	black seed
PP2	white seed	PP16	white seed
PP3	black seed	PP18	white seed
PP5	white seed	PP19	white seed
PP6	black seed	PP20	white seed
PP7	white seed	PP21	white seed
PP9	white seed		
PP10	black seed		
PP11	black seed		
PP12	black seed		
PP13	white seed		

EXHIBIT A

BREEDING HISTORY QUEEN OF HEARTS

In the spring of 1997, the surviving seventeen F2 lines were seeded near Corcoran, California. Trials of the F2 lines were concurrently evaluated in the Salinas Valley of California, and several of the lines were dropped prior to seed harvest maturity in August. In August of 1997, F3 seed was harvested from the following lines as follows:

1997

Selection	Black Seed	White Seed
PP3	1,2,3	4,5,6
PP6	1,2,3	4,5,6
PP10	1,2,3	4,5,6
PP11	1,2,3	4,5,6
PP12	1,2,3	4,5,6,7
PP15	1,2,4,5,6	3

The F3 generation of each of the six lines was segregating for seed color. It was decided at harvest to limit the numbers of each line to three selections of each color.

No trials were planted in Yuma, Arizona during the winter of 1997-1998.

Trials were conducted during the lettuce season of 1998 in the Salinas Valley of California to screen the 37 F3 lines. Lines were evaluated for corky root rot resistance, tipburn resistance, cupping, bolt tolerance, weight, internal color, and interior leaf fold.

The maternal plant Paragon PIC has a large, wide butt appearance with large core diameter. Color is medium green with a dull vanguard reflectance. Butt appearance of Presidio is medium in size, with a sharper, thinner midrib with a dark, shiny appearance. Segregation was noted in all lines.

Lines with the most desirable types from Salinas Valley trials were designated for seed harvest in August of 1998. All lines of the PP material were being produced concurrently in the San Joaquin Valley of California near Corcoran. Seed was harvested from designated lines as follows:

1998

I.D.	Selections	Seed Color
PP 6-1 -	1,2,3,4,5, Balance	all black seed
PP10-1 -	1,2,3,4,5, Balance	all black seed
PP11-5-	1,2,3,4,5,6,7,8,9,10, Balance	all white seed
PP12-5-	1,2,3,4,5,6,7,8,9,10, Balance	all white seed
PP15-6-}	1,2,3,4,5, Balance	black seed
	6,7,8,9,10,	white seed

EXHIBIT A

BREEDING HISTORY QUEEN OF HEARTS

Trials of 1998 harvested samples were planted in Yuma, Arizona and selection work continued for desirable traits. Spring and summer trials were conducted in the Salinas Valley of California. At this time selection work narrowed down three lines which were corky root rot resistant, and showed the promise for hearts production.

1999

I.D.	Selections	Seed Color
PP11-5-3-	1,2,3,4,5,6	all white seed
PP12-5-1-	1,2,3,4,5, Balance	all white seed
PP12-5-10-	1,2,3,4,5, Balance	all white seed

After seed harvest, the 1999 planting samples were prepared and planted in Yuma, Arizona, Winterhaven, California and near Calipatria, California. Lines were further screened for cupping, bolt tolerance, internal color, butt appearance and suitability to carton production as well as suitability for "heart" production.
The PP12-5-1 and PP12-5-10 selections were corky root rot resistant, and had the height, color, cupping and hearting style that were desired from this cross.

In the summer of 2000, a small increase of the composite line PP12-5-1- (1,2,4,5) was produced near Corcoran, California and harvested on August 04, 2000. After harvest designation of the increase was designated PP125. Production of the breeding lines also continued with individual plant selections of the individual lines for appraisal in trial plantings.

2000

I.D.	Selections	Seed Color
PP12-5-1-2	1,2,3,4,5, Balance	all white seed
PP12-5-1-Bal-	1,2,3,4,5, Balance	all white seed
PP12-5-10-2-	1,2,3,4,5, Balance	all white seed
PP12-5-10-50	1,2,3,4,5, Balance	all white seed

In the fall of 2000, trials were seeded in Yuma, Arizona of the breeding lines as well as plot trials of the PP125 production in commercial grower fields. Trials indicated a high level of uniformity of type in the 5-1-2 and 5-10-50 selections.

2001

I.D.	Seed Color
PP12-5-10-50-1-Mass	white seed
PP12-5-10-50-4-Mass	white seed
PP12-5-10-50-5-Mass	white seed
PP125-1050	composite for commercial production

6

EXHIBIT A

BREEDING HISTORY QUEEN OF HEARTS

It was the 5-10-50 lines that were selected for commercial seed production in the summer of 2001 near Corcoran, California by Paragon Seed, Inc. This commercial increase of the composite PP 125-10-50- (1,4,5) was planted on April 13, 2001 and harvested on August 14, 2001. This composite was designated PP 125-1050. This composite is very similar to the PP125 line produced in crop season 2000, however the uniformity to type and slight cupping appeared better in the stock seed provided for production in 2001.

Uniformity to type was determined at harvest maturity in June 2001 near Corcoran, California. No off types or variants were noted in this production.

Growouts of the stock seed in the Salinas Valley also confirmed uniformity to type was very good, meeting all commercially acceptable standards. No off types or variants were noted in these trials. Commercial trials with growers and seed dealers continued throughout 2001 using the PP125 designation.

Queen of Hearts was developed using five generations of single plant selections followed by a single generation of mass selection.

Queen of Hearts has been observed for three generations of reproduction and during the seed increase period and is stable and uniform. No variants were observed.

Exhibit B

Novelty Statement Queen of Hearts

'Queen of Hearts' is a tall, large framed romaine lettuce variety developed specifically for "hearts" processing in the food service industry.

The type of romaine best suited for "hearts" is one that is tall and cylindrical in nature, slow bolting with a low core, with a very yellow interior color. The leaves should be thick to provide good texture and weight as outer wrapper leaves are stripped to reveal only the interior of the plant. Normally, approximately fifty percent of the outer leaves are removed from the plant to make the delicate "hearts" product. Slight cupping of the mature plant at harvest time is important to protect the interior growing point, yet development of the dark interior green color.

'Queen of Hearts' is most similar to the variety 'Green Towers'; however, 'Queen of Hearts' is taller than 'Green Towers' at harvest maturity. (35.3 cm's vs. 33.4 cms).

'Queen of Hearts' is most similar to the variety 'Green Towers'; however, the average weight of a "head" of 'Green Towers' is heavier than 'Queen of Hearts'. (495 gm's vs. 525 gm's.)

'Queen of Hearts' is most similar to the variety 'Green Towers'; however, 'Queen of Hearts' is slower bolting and has a lower core height than 'Green Towers'. (2.3 in. vs. 2.5 in.)

'Queen of Hearts' is resistant to Corky Root Rot whereas 'Green Towers' is susceptible to Corky Root Rot. Corky root (CR) is caused by the bacterium *Rhizomonas suberfaciens* (gen.nov,sp.nov.) (van Bruggen el al., 1990). The common strain found in California is identified as CA 1. The resistance is conferred by a single recessive gene (cor cor) (Brown and Michelmore, 1988).

'Green Towers' has been the industry standard for many years as the standard for "carton" romaine lettuce. 'Green Towers' exhibits excellent uniformity, large plant size, and packs well into cartons. 'Queen of Hearts' develops a longer, more cylindrical romaine plant, and is not as well suited for carton pack. The cylindrical nature of 'Queen of Hearts' makes this variety well adapted to a food service "hearts" product.

Exhibit B

Novelty Statement Queen of Hearts

There are several styles of packing "hearts", but on average, the weight of a "heart" is approximately 100 to 125 grams with a leaf count of fifteen to twenty leaves. A head of mature carton romaine lettuce may contain 30 to 35 leaves with a weight of 500 to 600 grams.

'Queen of Hearts' is most similar to 'Green Towers', however, there are several distinct differences:

	'Queen of Hearts'	**'Green Towers'**
Cotyledon leaf shape	Broad	Spatulate
Cotyledon leaf undulation	Slight	Flat
Mature leaf incision depth	Absent	Moderate
Mature leaf indentation	Entire	Crenate
Leaf blistering	Smooth	Moderate
Butt Shape	Rounded	Slightly concave
Butt Midrib	Moderately Raised	Prominently raised
Corky Root Resistance	Resistant CA1 (cor cor) gene	Susceptible

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE DIVISION

EXHIBIT C

OBJECTIVE DESCRIPTION OF VARIETY
LETTUCE *Lactuca sativa*

NAME OF APPLICANT (S)
Paragon Seed, Inc.

ADDRESS (Street and No. or R.F.D. No., City, State, and ZIP Code)
P.O. Box 1906
Salinas, California 93902-1906

FOR OFFICIAL USE ONLY
PVPO NUMBER
200200029
VARIETY NAME
Queen of Hearts
EXPERIMENTAL DESIGNATION
PP 125

Place numbers in the boxes for the characters which best describe this variety. Measured data should be the mean of an appropriate number (at least 10) of well spaced plants. Royal Horticultural Society or any recognized color standard may be used to determine plant colors.

The location of the test area is: Salinas, California

Color System Used: Royal Horticultural Chart

1. PLANT TYPE: *(See list of suggested check varieties page 4.)*

[0 4]

01=Cutting/Leaf
02=Butterhead
03=Bibb
04=Cos or Romaine
05=Great Lakes Group
06=Vanguard Group
07=Imperial Group
08=Eastern (Ithaca) Group
09=Stem
10=Latin
11=OTHER

2. SEED:

COLOR: [1] 1=White (Silver Gray) 2=Black (Gray Brown) 3=Brown (Amber)

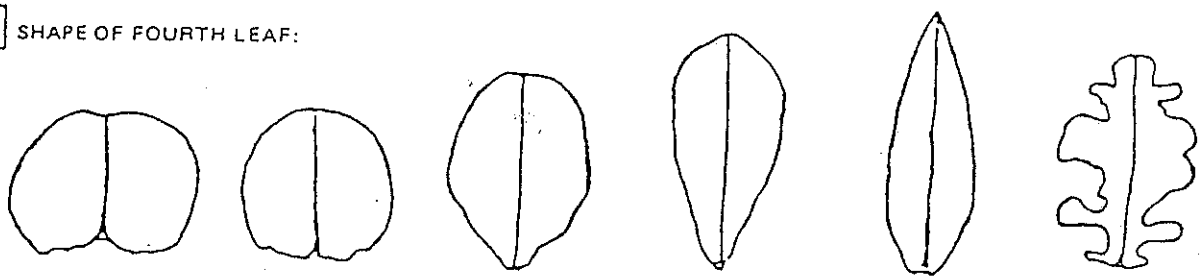
LIGHT DORMANCY: [2] 1=Light Required 2=Light Not Required

HEAT DORMANCY: [1] 1=Susceptible 2=Not Susceptible

3. COTYLEDON TO FOURTH LEAF STAGE: NOTE: Provide a color photograph or photocopy of the fourth leaf from 20 day old seedling grown under optimal conditions.

[1] SHAPE OF COTYLEDONS: 1=Broad 2=Intermediate 3=Spatulate

[4] SHAPE OF FOURTH LEAF:



[2 0] LENGTH/WIDTH INDEX OF FOURTH LEAF: L/W x 10

[1] APICAL MARGIN:
[5] BASAL MARGIN:
1=Entire 2=Crenate/Gnawed 3=Finely Dentate 4=Moderately Dentate 5=Coarsely Dentate 6=Incised 7=Lobed 8=OTHER *(specify)*

[2] UNDULATION: 1=Flat 2=Slight 3=Medium 4=Marked

[4] GREEN COLOR: 1=Yellow Green 2=Light Green 3=Medium Green 4=Dark Green 5=Blue Green 6=Silver Green 7=Gray Green

ANTHOCYANIN:

[1] DISTRIBUTION: 1=Absent 2=Margin Only 3=Spotted 4=Throughout 5=OTHER *(specify)*

[0] CONCENTRATION: 1=Light 2=Moderate 3=Intense

[1] ROLLING: 1=Absent 2=Present

[1] CUPPING: 1=Uncupped 2=Slight 3=Markedly

[1] REFLEXING: 1=None 2=Apical Margin 3=Lateral Margins

4. MATURE LEAVES *(observe harvest-mature outer leaves)*:

NOTE: Provide color photo of harvest-mature leaves which accurately shows color and margin characteristics.

MARGIN:

[1]	INCISION DEPTH: *(deepest penetration of the margin)*	1=Absent/Shallow (Dark Green Boston)	2=Moderate (Vanguard)	3=Deep (Great Lakes 659)
[1]	INDENTATION: *(finest divisions of the margin)*	1=Entire (Dark Green Boston) 2=Shallowly Dentate (Great Lakes 65)	3=Deeply Dentate (Great Lakes 659) 4=Crenate (Vanguard)	5=OTHER *(specify)*
[1]	UNDULATION OF THE APICAL MARGIN:	1=Absent/Slight (Dark Green Boston)	2=Moderate (Vanguard)	3=Strong (Great Lakes 659)
[4]	GREEN COLOR:	1=Very Light Green (Bibb) 2=Light Green (Minetto)	3=Medium Green (Great Lakes) 4=Dark Green (Vanguard)	5=Very Dark Green 6=OTHER

ANTHOCYANIN *(grown at or below 10 C)*:

[1]	DISTRIBUTION:	1=Absent 2=Margin Only (Big Boston)	3=Spotted (Calif. Cream Butter) 4=Throughout (Prize Head)	5=OTHER *(specify)*
[0]	CONCENTRATION:	1=Light (Iceberg)	2=Moderate (Prize Head)	3=Intense (Ruby)
[3]	SIZE:	1=Small	2=Medium	3=Large
[3]	GLOSSINESS:	1=Dull (Vanguard)	2=Moderate (Salinas)	3=Glossy (Great Lakes)
[1]	BLISTERING:	1=Absent/Slight (Salinas)	2=Moderate (Vanguard)	3=Strong (Prize Head)
[3]	LEAF THICKNESS:	1=Thin	2=Intermediate	3=Thick
[1]	TRICHOMES:	1=Absent (smooth)	2=Present (spiny)	

5. PLANT *(at market stage. Choose a comparison variety appropriate for this type.)*:

SPREAD OF FRAME LEAVES:
[2][0] cm This Variety [2][4] cm Green Towers *(specify comparison variety)*

HEAD DIAMETER *(market trimmed with single cap leaf)*:
[ ][ ] cm This Variety [ ][ ] cm _____ *(specify comparison variety)*

[5]	HEAD SHAPE:	1=Flattened 2=Slightly Flattened	3=Spherical 4=Elongate	5=Non-Heading 6=OTHER
[3]	HEAD SIZE CLASS:	1=Small	2=Medium	3=Large
[4][8]	HEAD COUNT PER CARTON			

HEAD WEIGHT:
[4][7][2] g This Variety [4][9][4] g Green Towers *(specify comparison variety)*

[1]	HEAD FIRMNESS:	1=Loose 2=Moderate	3=Firm 4=Very Firm

6. BUTT *(bottom of market-trimmed head)*:

[3]	SHAPE:	1=Slightly Concave	2=Flat	3=Rounded
[2]	MIDRIB:	1=Flattened (Salinas)	2=Moderately Raised	3=Prominently Raised (Great Lakes 659)

7. CORE *(stem of market-trimmed head)*:

[4][3] mm Diameter at base of head

[ ][ ].[ ] Ratio of head diameter/core diameter

Core height from base of head to apex:
[5][8] mm This Variety [6][2] mm Green Towers *(specify comparison variety)*

8. BOLTING *(Give First Water Date 4/15/01)*: NOTE: First Water Date is the date seed first receives adequate moisture to germinate. This can and often does equal the planting date.

Number of days from First Water Date to seed stalk emergence *(summer conditions)*:
[ ][6][9] This Variety [ ][6][9] Hearts Delight *(specify comparison variety)*

[3]	BOLTING CLASS:	1=Very Slow 2=Slow	3=Medium 4=Rapid	5=Very Rapid

Height of mature seed stalk:
[1][0][5] cm This Variety [1][0][9] cm Hearts Delight *(specify comparison variety)*

11

BOLTING cont'd. on next page

200200029

Spread of Bolter Plant *(at widest point)*:

[2][8] cm This Variety [3][2] cm Green Towers *(specify comparison variety)*

[1] BOLTER LEAVES: 1=Straight 2=Curved

[1] MARGIN: 1=Entire 2=Dentate

[2] COLOR: 1=Light Green 2=Medium Green 3=Dark Green

BOLTER HABIT:

[2] TERMINAL INFLORESCENCE: 1=Absent 2=Present

[2] LATERAL SHOOTS: *(above head)* 1=Absent 2=Present

[1] BASAL SIDE SHOOTS: 1=Absent 2=Present

9. MATURITY *(earliness of harvest-mature head formation)*:

NOTE: Complete this section for at least one season.

SEASON	Applic. 1/ #of days	Check 1/ #of days	CHECK VARIETY 2/
Spring	102	103	Grand Prize
Summer	062	063	Frontier
Fall	062	060	Green Forest
Winter	069	070	Green Towers

Give planting date(s), and location(s):

Spring	King City, Ca.	plant	01/18/01	Harvest	05/03/01
Summer	Chualar, Ca.	plant	05/16/01	Harvest	07/18/01
Fall	Salinas, Ca.	plant	07/19/01	Harvest	09/18/01
Winter	Yuma, Arizona	plant	09/27/00	Harvest	12/05/00

1/ First water date to harvest. 2/ Fill in check variety name on the appropriate line.

10. ADAPTATION:

PRIMARY REGIONS OF ADAPTION *(tested and proven adapted)*: (0=Not tested 1=Not Adapted 2=Adapted)

[2] Southwest *(Calif., Ariz. desert)* [2] West Coast [0] Northeast

[0] Northcentral [0] Southeast [0] OTHER _____

SEASON:

[2] Spring (area Yuma, Az Salinas, Ca [2] Fall (area Salinas, Santa Maria CA.

[2] Summer (area Salinas, Santa Maria CA [2] Winter (area Yuma, Az Imperial Valley CA

[0] GREENHOUSE: 0=Not tested 1=Not Adapted 2=Adapted

[1] SOIL TYPE: 1=Mineral 2=Organic 3=Both

12

200200029

11. DISEASES AND STRESS REACTIONS (0=Not tested; 1=Susceptible; 2=Intermediate; 3=Resistant; 4=Highly resistant; 5=Tolerant):

RAP 7/6/05

CA-I

VIRUS

- [1] Big Vein
- [1] Lettuce Mosaic
- [0] Cucumber Mosaic
- [0] Broad Bean Wilt
- [0] Turnip Mosaic
- [0] Beet Western Yellows
- [0] Lett. Infectious Yellows
- [0] Other Virus _____

FUNGAL/BACTERIAL

- [4] Corky Root Rot (Pythium Root Rot)
- [0] Downy Mildew (Races _____)
- [0] Powdery Mildew
- [1] Sclerotinia Rot
- [0] Bacterial Soft Rot (Pseudomonas spp. & others)
- [0] Botrytis (Gray Mold)
- [0] OTHER _____

INSECTS

- [1] Cabbage Loopers
- [0] Root Aphids
- [1] Green Peach Aphid
- [0] Other Insect _____

PHYSIOLOGICAL/STRESS

- [2] Tipburn
- [5] Heat
- [0] Drought
- [0] Cold
- [0] Salt
- [0] Brown Rib (Rib Discoloration, Rib Blight)
- [0] OTHER _____

POST HARVEST

- [0] Pink Rib
- [0] Russet Spotting
- [0] Rusty Brown Discoloration
- [0] Internal Rib Necrosis (Blackheart, Gray Rib, Gray Streak)
- [0] Brown Stain

12. BIOCHEMICAL OR ELECTROPHORETIC MARKERS:

13. COMMENTS:

SUGGESTED CHECK VARIETIES

TYPE	CHECK VARIETY
1) CUTTING/LEAF	SALAD BOWL
2) BUTTERHEAD	DARK GREEN BOSTON
3) 8188	8188
4) COS. OR ROMAINE	PARRIS ISLAND
5) GREAT LAKES GROUP	GREAT LAKES 659-700
6) VANGUARD GROUP	VANGUARD
7) IMPERIAL GROUP	VIVA
8) EASTERN GROUP	ITHACA
9) STEM	CELTUCE
10) LATIN	MATCHLESS

13

Paragon Seed, Inc.

QUEEN OF HEARTS

QUEEN OF HEARTS GREEN TOWERS

Note : Photocopy of fourth leaf from 20 day old plant grown under optimum conditions.

Paragon Seed, Inc. Merrill Farms Spreckels Ranch 6/98

Paragon PIC (front 3 heads) Presideo (2 heads rear)

Paragon PIC (front) Presideo (center) Clemente (rear)

Paragon Seed, Inc. Williams Ranch lot 64 6/14/00

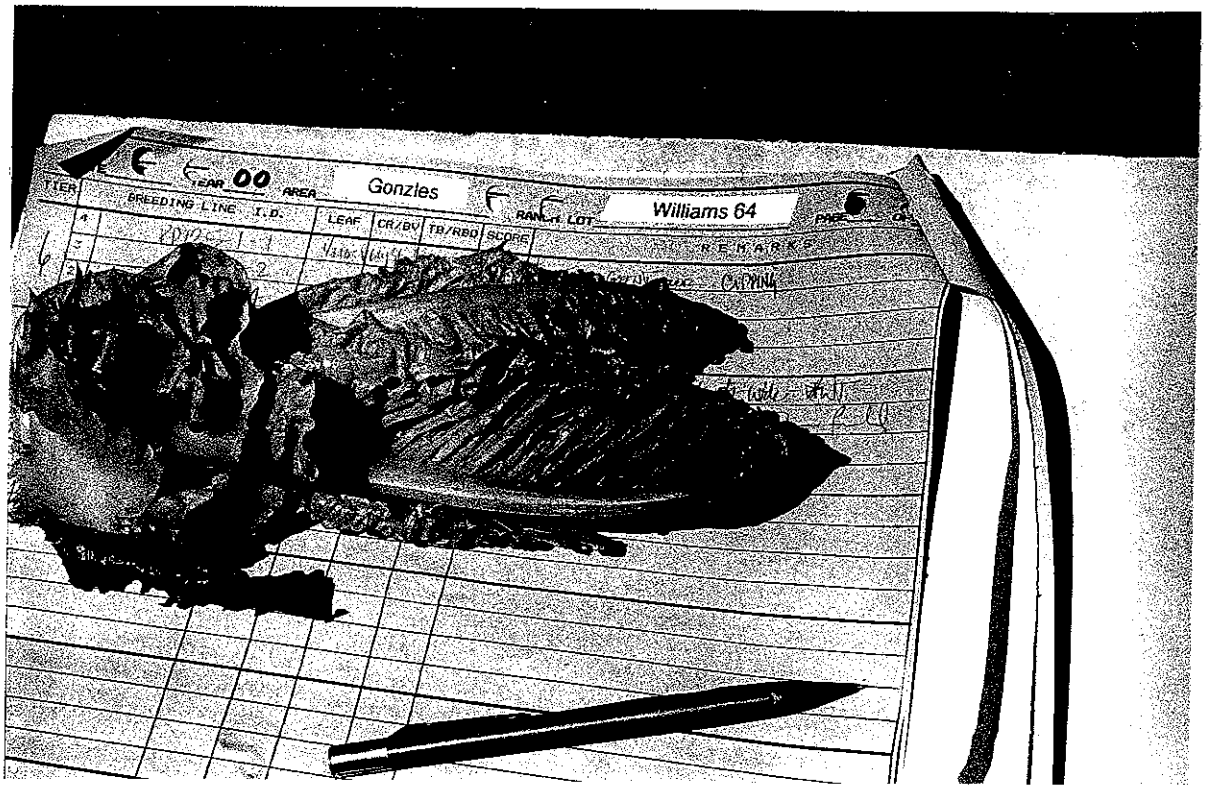
PP 125 QUEEN OF HEARTS

PIC a/g

PARAGON SEED ALTURA

Paragon Seed , Inc. Williams Ranch lot 64 6/14/00

Hearts Delight



Tipburn

Experimental PP 125 Queen of Hearts

Exp. PP 125
Queen of Hearts

Field Planting Green Towers

Field planting Green Towers Corky Root Rot susceptible

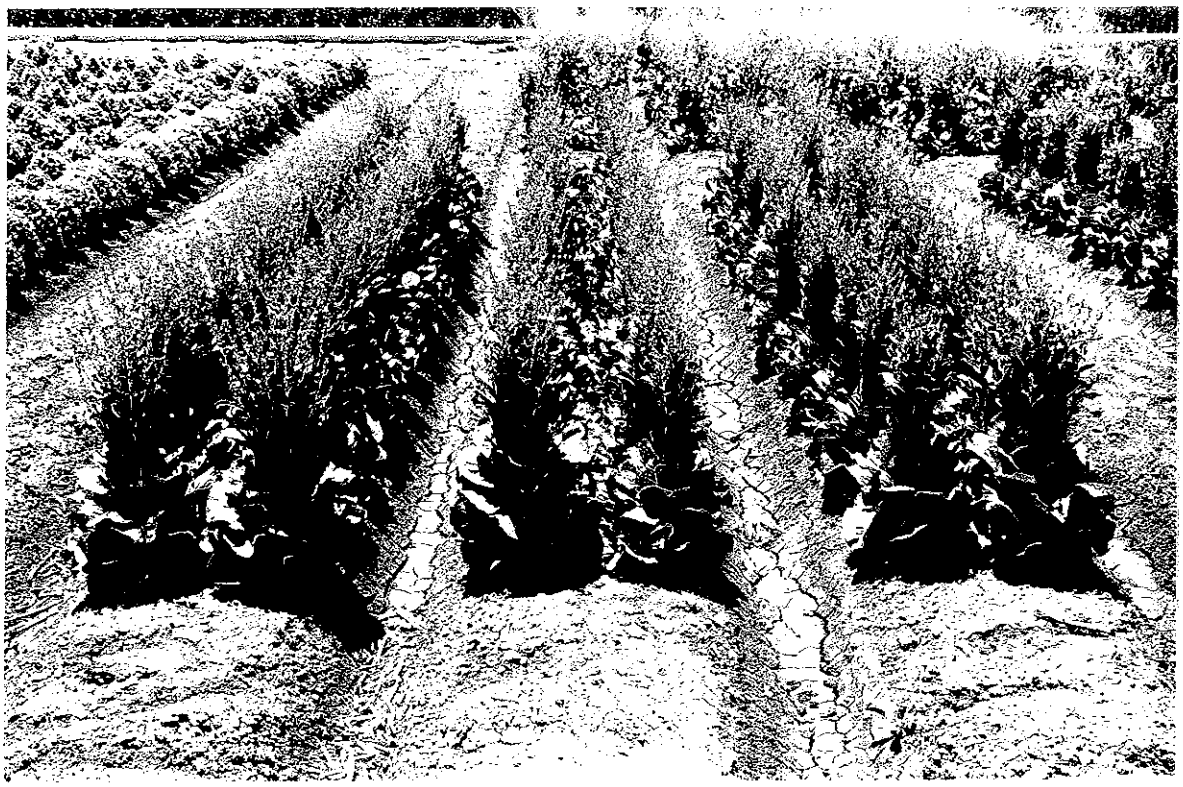
Salinas Valley, California Blanco Farms July 9, 2000 200200029

Trial looking north (l to r) C381 PC12M2 PP125 PIC a/g

FIELD

Trial looking south (l to r) PIC a/g PP125 PC12M2 C381
Field planting in foreground Green Towers

Paragon Seed, Inc. San Joaquin Valley June 2001



23C2 – 5 - 911

Queen of Hearts

Paragon Seed, Inc.

San Joaquin Valley June 2001

Altura PIC a/g Exp PC12M2 PP 125 Queen of Hearts

Queen of Hearts Exp PP 125

12

Hearts Delight

Mardi Gras

200200029

PARAGON SEED COMPANY

P.O. Box 1906 Salinas, Ca. 93902 831-753-2100

Queen of Hearts vs Green Towers

Grown on Blanco Farms Salinas, California Harvest date -: July 10, 2000

	Queen Hearts	Green Towers	Queen Hearts	Green Towers	Queen Hearts	Green Towers	Queen Hearts	Green Towers
	Height	Height	Core Dia	Core Dia	Weight	Weight	Core Ht	Core Ht
Count	24	24	24	24	24	24	24	24
Sum	847.0	801.5	1,036.0	1,081.0	11,875.0	12,589.0	55.00	59.25
Mean	35.29	33.40	43.17	45.04	494.79	524.54	2.29	2.47
Maximum Value	37.0	35.0	45.0	46.5	525.0	546.0	2.75	2.75
Minimum Value	34.0	32.0	40.0	43.0	475.0	506.0	1.75	2.00
Variance	0.63	0.48	1.97	0.91	221.04	70.52	0.06	0.05
Std.Dev	0.79	0.69	1.40	0.95	14.87	8.40	0.25	0.21
Joint Variance	*****	0.55	*****	1.44	*****	145.78	*****	0.05
Jt Deg of Freedom	*****	46	*****	46	*****	46	*****	46.00
t-Test Parameter	*****	8.830	*****	5.411	*****	8.535	*****	2.63
Level of Significance	*****	.0000	*****	.0000	*****	.0000	*****	.0115
Confidence Level %	*****	100.000	*****	100.000	*****	100.000	*****	98.85
	CM'S	CM's	MM's	MM'S	Grams	Grams	Inches	Inches
MEASUREMENTS FOR SAMPLES	35.0	33.0	43.0	45.0	499	526	2.50	2.50
	35.0	33.5	42.0	46.0	512	524	2.50	2.50
	36.0	34.0	45.0	43.0	524	530	2.75	2.75
Solidity measured on a scale of 1 to 5	34.0	33.0	43.0	45.0	488	520	2.50	2.50
	36.0	33.5	42.0	46.0	500	524	2.25	2.50
	35.0	32.0	45.0	44.0	488	546	2.50	2.75
	35.0	34.5	43.0	46.0	499	525	2.25	2.50
Note: The Level of Significance is determined by using Excel 5's 2-tail type 2 built in T-test function directly over the ranges of data.	35.0	33.5	40.0	46.5	485	533	2.00	2.50
	36.0	34.0	44.0	45.0	525	520	2.50	2.75
	37.0	33.0	43.0	46.5	475	515	2.50	2.50
	35.0	33.0	40.0	45.0	485	525	2.50	2.25
	35.0	33.5	43.0	44.0	490	530	2.00	2.00
	36.0	34.0	43.0	46.0	485	525	2.50	2.50
	34.0	33.5	45.0	45.0	480	540	2.00	2.25
	36.0	33.0	42.0	44.0	475	523	2.25	2.50
	35.0	33.0	45.0	45.0	500	526	2.25	2.75
	34.0	34.0	43.0	43.5	510	525	1.75	2.50
	36.0	33.0	44.0	44.0	495	516	2.00	2.75
	35.0	32.0	44.0	46.0	480	511	2.25	2.50
	36.0	35.0	43.0	45.0	475	530	2.50	2.25
	36.5	34.0	45.0	46.0	500	524	2.50	2.50
	35.0	33.0	43.0	45.0	490	506	2.25	2.50
	34.5	33.0	42.0	45.0	495	525	2.00	2.25
	35.0	33.5	44.0	44.5	520	520	2.00	2.00

24

PARAGON SEED COMPANY

P.O. Box 1906 Salinas, Ca. 93902 831-753-2100

Queen of Hearts vs Hearts Delight

Grower MKM Ranches, Salinas Ca. Harvest August 11, 2001

	Queen of Hearts Height	Hearts Delight Height	Queen of Hearts Core dia	Hearts Delight Core dia	Queen of Hearts Weight	Hearts Delight Weight	Queen of Hearts Core Ht	Hearts Delight Core Ht
Count	24	24	24	24	24	24	24	24
Sum	865.0	840.0	1,075.0	1,077.0	12,625.0	12,890.0	64.25	74.00
Mean	36.04	35.00	44.79	44.88	526.04	537.08	2.68	3.08
Maximum Value	37.5	36.0	46.0	46.0	540.0	560.0	3.00	3.50
Minimum Value	34.0	34.0	44.0	44.0	510.0	525.0	2.00	2.75
Variance	0.72	0.26	0.52	0.46	41.26	104.17	0.07	0.03
Std.Dev	0.85	0.51	0.72	6.00	6.42	10.21	0.27	0.18
Joint Variance	*****	0.49	*****	0.49	*****	72.71	*****	0.05
Jt Deg of Freedom	*****	46	*****	46	*****	46	*****	46.00
t-Test Parameter	*****	5.164	*****	0.412	*****	4.486	*****	6.17
Level of Significance	*****	.0000	*****	.6823	*****	.0000	*****	.0000
Confidence Level %	*****	99.999	*****	31.774	*****	99.995	*****	100.00
	CM'S	CM'S	MM'S	MM'S	Grams	Grams	Inches	Inches
MEASUREMENTS	37.0	35.0	44.0	44.0	530	525	2.50	3.00
FOR	36.5	36.0	46.0	45.0	525	530	3.00	3.00
SAMPLES	36.0	35.0	44.0	46.0	535	550	3.00	3.00
	37.0	35.0	45.0	45.0	540	525	2.50	3.25
Solidity measured	36.5	35.0	45.0	44.0	525	535	2.75	3.00
on a scale of	36.0	35.0	45.0	44.0	530	540	2.00	3.00
1 to 5	35.5	35.5	44.0	46.0	525	560	2.50	3.25
	37.0	35.0	46.0	45.0	520	555	3.00	3.50
Note:	36.0	36.0	44.0	45.0	530	545	2.75	3.25
The Level of	37.5	35.0	44.0	44.0	520	535	3.00	3.00
Significance is	36.0	35.0	45.0	44.0	525	540	2.50	3.00
determined by	36.5	34.5	45.0	45.0	525	535	2.50	3.00
using Excel 5's	34.0	34.5	44.0	45.0	525	540	3.00	3.25
2-tail type 2	36.0	34.5	45.0	45.0	530	550	2.50	3.00
built in T-test	35.5	35.0	45.0	45.0	530	525	2.50	3.00
function directly	36.0	35.0	44.0	44.0	520	545	2.50	2.75
over the	36.5	35.0	46.0	46.0	530	530	2.75	3.00
ranges of data.	34.5	36.0	44.0	45.0	530	525	3.00	3.00
	36.0	35.0	45.0	45.0	515	540	3.00	3.00
	35.5	35.0	45.0	46.0	520	525	2.50	3.50
	36.0	35.0	45.0	45.0	530	540	2.50	3.25
	34.5	34.0	45.0	45.0	525	530	3.00	3.00
	36.0	35.0	46.0	45.0	510	540	2.50	3.00
	37.0	34.0	44.0	44.0	530	525	2.50	3.00

ATTACHMENT A

Vegetable Cultivar Descriptions for North America

Lettuce (M-Z), Lists 1-26 Combined

Edited by Edward J. Ryder and James D. McCreight

U.S. Agricultural Research Station
1636 East Alisal Street
Salinas, CA 93905

Major Cos - Breeder and vendor: Seminis Vegetable Seeds-Genecorp. Characteristics: 77 days to maturity, head heavy, upright, dark green, lightly blistered leaves, early, slow bolting, heavy, compact heads with wide, well colored leaves. 1991.

Manavert - Breeder and vendor: Enza Zaden. Adaptation: North America. 1997.

Marin (BOS 9041) - Vendor: Orsetti Seed Co. Characteristics: a somewhat larger Two Star type green leaf with improved color; leaves are wavy, frilled, have heavy weight, and thick texture; excellent pliability is beneficial to retaining head integrity during packing and shipping. Similar: Two Star. Adaptation: U.S. PVP pending. 2001.

Marksman - Breeder and vendor: Petoseed. Characteristics: crisphead type, large frame, dark green color. Resistance: downy mildew pathotypes 1, 2, 3, 4. Similar: El Toro. 1993.

Marquis - Breeder and vendor: Asgrow. Characteristics: cos type, thick, dark green blistered leaves, good flavor. Resistance: lettuce mosaic virus, Bidens mottle virus, corky root rot, tipburn, thermodormancy. Similar: Parris Island Cos. 1991.

Meadow - Breeder and vendor: Enza Zaden.

Merit - Breeder: USDA, Beltsville, Md. Parentage: Slobolt x complex hybrid selection. Similar: Great Lakes. Characteristics: slow bolting and excellent butt conformation. Resistance: big vein. Adaptation: Great Lakes area. 1958.

Merit 88 - Breeder: USDA. Vendor: Petoseed. Characteristics: crisphead type, large frame and head, medium leaf thickness, dark green leaves, ribs similar to Merit, black seed. Resistance: pink rib, tipburn, bolting, big vein. Resistance: tolerance to heat, frost. Similar: Merit.

Meritmore - Breeder: Orsetti. Vendor: Orsetti. Characteristics: crisphead type, medium to

large frame, head and butt, green leaves, tan seed. Resistance: bolting, tipburn, downy mildew California pathovar 1, big vein; tolerance to heat, frost. Similar: Merit. 1994.

Mesaverde (no. 3285) - Breeder and vendor: Keystone Seed Co. Parentage: Mesa 659 x Calmar BC5-S2. Characteristics: open pollinated. Resistance: downy mildew (race 5). Similar: Mesa 659. Adaptation: western United States. 1977.

Midas - Breeder: USDA, Beltsville, Md. Parentage: Slobolt x Unrivaled. Similar: White Boston. Adaptation: wide. 1954.

Midget Cos - Breeder: Pieters Wheeler, Gilroy, Calif. Characteristics: very early dwarf Cos type, processing cabbage type leaf quality; very sweet. 1965.

Militia (SSC 1349) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: medium wine-red oak leaf lettuce; it produces moderately broad, multi-lobed leaves with a wavy apperance and smooth leaf margins; smooth, pliable texture. Resistance: downy mildew (pathotypes I, IIA, IIB, III, IV, V). Similar: Armada. Adaptation: U.S. lettuce growing regions. 2004.

Minetto (23IV-9) - Breeder: Cornell Univ., Ithaca, N.Y. Characteristics: small growing, slow bolting summer cultivar that will pack 24 to the carton if grown on good muck. Veg. Crops Mimeo. VC-122 June, 1964.

Mini-Green - Breeder: W. Waycott and E.J. Ryder. Vendor: USDA Res. Sta., Salinas, CA. Characteristics: iceberg type, heads size of a baseball, dark green color with solid heads, interior color is creamy yellow, takes heat well.

Mirage - Breeder and vendor: Quali-Sel, Inc. Characteristics: F2 hybrid; exceptional bolt tolerance, uniformity for heading and ability to make heads in hot weather. Resistance: excellent tolerance to tipburn. Similar: Oswego. Adaptation: early fall planting in Arizona and California. 1979.

Mission - Breeder and vendor: Quali-Sel, Inc. Characteristics: open pollinated; short core length, frilly leaf margins, and solid heads. Resistance: none claimed. Similar: Great Lakes Mesa 659. Adaptation: Colorado and southern Salinas Valley. PVP Certificate No. 7900084. 1979.

Misty Day - Breeder: E.J. Ryder and W. Waycott. Vendor: USDA, ARS; Salinas, California. Characteristics: crisphead type, dull, dark green outer leaves slightly more pale than Salinas, green color extends close to the core, leaf margins mildly undulate, moderately dentate, strongly ruffled, texture soft, surface crinkled or blistered, outer leaves broader than long, interior creamy yellow, heads firm to hard, flat ribs and butt, black seed. Resistance: corky root; susceptible to bolting induced by high temperature, lettuce mosaic virus, big vein. 1991.

Mohawk (PS 6386 MI) - Breeder: Doug Sousa. Vendor: Seminis Vegetable Seeds-Petoseed. Parentage: open pollinated. Characteristics: Vanguard type crisphead, early maturity, sure heading, large head, large wrap, dark green color, bolt tolerant, plant late September to early October. Similar: Vanguard. Adaptation: desert southwest U.S. 1998.

20020029

Mojave - Breeder and vendor: Harris Moran. Characteristics: crisphead type, very large frame, highly uniform, slow bolting, very dark dull green leaves. Resistance: lettuce mosaic virus, downy mildew pathotype 3. Similar: var. Vanguard.

Monolith (SSC 30375) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Resistance: big vein, downy mildew (races 3, 5). Similar: Winterhaven. Adaptation: during winter in the desert Southwest. 2001.

Montello - Breeder: L. Sequeira. Vendor: Univ. Wisconsin. Characteristics: 75 days to maturity, large, dark green heads, highly tolerant to corky root, heat, and splitting; vigorous, widely adapted. 1978.

Montemar (E 9201) - Breeder and vendor: Ferry-Morse Seed Co., Inc. Characteristics: earlier with uniform slightly smaller frame and larger grayer-green head than Calmar. Similar: Calmar. 1971.

Monterey (67-345-4-6, 10, 13, 17) - Breeder: USDA, Salinas, Calif. Parentage: USDA No. 8830 x Calmar F6. Characteristics: early, uniform, crisphead type for mid and late summer. Similar: Calmar. Resistance: downy mildew; tolerance to tipburn. Adaptation: central Coast valleys of California. 1973.

Mor 109 - Breeder: Select Seed of California. Vendor: Petoseed. Characteristics: crisphead type, large frame and head, thick dark green leaves, black seed. Resistance: pink rib, tipburn, bolting, downy mildew California pathovar 3; tolerance to heat, frost. Similar: Vanguard. 1985.

Morangold (XP3) - Breeder and vendor: Moran Seeds. Parentage: selection from Cal K-60. Characteristics: open pollinated; most concentrated of Calmar types, few days earlier with better color, head cover, higher yields than other Calmar types. Resistance: tipburn and downy mildew. Similar: Cal K-60. Adaptation: all seasons in California coastal valleys, fall plantings in desert areas. PVP Certificate No. 7600039. 1976.

Moranguard - Breeder and vendor: Moran Seeds. Parentage: single-plant selection from Vanguard. Characteristics: open pollinated; large heads, dark dull green color, slower bolting than Vanguard. Similar: Vanguard. Adaptation: desert and inland valleys for fall planting. 1969.

Mossberg (SSC 30387) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Resistance: big vein. Similar: Pam's Island Cos. Adaptation: all major lettuce growing regions. 2000.

Mt. Signal - Breeder: Brinker-Orsetti. Vendor: Brinker. Characteristics: crisphead type, large size, minimal cover. Similar: Vanmax. 1990.

Murietta - Breeder and vendor: Ferry-Morse. Characteristics: crisphead type, attractive shape and butt appearance, excellent processed color and texture. Resistance: tipburn, downy mildew pathotype 3. Similar: Vanguard.

Mustang - Breeder and vendor: Coastal. Characteristics: crisphead type, medium to large

frame and head, smooth ribs, black seed. Resistance: downy mildew California pathovars 1, 2A, 3; big vein. 1994.

Nancy - Vendor: Seedway, Inc. Characteristics: 65 days to maturity, medium head, compact, good weight, smooth, rounded leaves, medium green color, excellent quality and texture and clean underside.

Navigator (G x 615) - Breeder: Jodi Grossen. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: Vanguard type, dark green, good head formation. Similar: Annie. Adaptation: late fall, southwest U.S. 1998.

Nevada - Vendor: Vilmorin. Characteristics: broad shiny leaves, crunchy texture, bright medium green. Resistance: mildew R11, lettuce mosaic virus. Similar: Salvina, Masaida. 1991.

New Dominion - Breeder: Quali-Sel. Vendor: Synergene. Characteristics: crisphead type, large frame and head, thick dull dark green and crisp leaves, large butt, mid-winter harvest in desert southwest United States, dark brown seed. Resistance: bolting, tipburn; tolerance to frost. Similar: Vanguard. 1993.

New Red Fire - Characteristics: 48 days to maturity, medium to large, dark red heads, red tinge, leaves ruffled with waxy margins, red loose leaf with full center, uniform head holds color well and slow bolting.

New York PW55 - Breeder and vendor: Pieters-Wheeler Seed Co., Gilroy, Calif. Parentage: continued selection since 1927 out of New York. Similar: small New York type. Characteristics: medium, early whirl type coverage. Resistance: tipburn. Adaptation: northeast, midwest, and northwest. 1942.

Nile (LM 2641) - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: green butter lettuce; 45-55 day maturity; medium head size; medium green color; smooth thick leaves; good shelf life. Resistance: downy mildew. Similar: Boston Bibb. Adaptation: can be cultivated in U.S. if sown in proper season.

Niner - Breeder and vendor: Asgrow. Characteristics: crisphead type, good heading ability, good dark green color, late Empire and early Merit slot in the desert southwest United States, and Empire slot in the San Joaquin Valley, California. Similar: Merit. 1994.

No. 456 (Cornell 456, Imperial 456) - Breeder: Cornell Univ., Ithaca, N.Y., and Hort. Field Station, USDA, La Jolla, Calif. Parentage: Imp. 152 x Brittle Ice, backcrossed to Imp. 152, selected for eight generations. Characteristics: medium to small size, globular, exposed heads, frame leaves with glossy green color. Resistance: brown blight; some resistance to tipburn. Similar: Great Lakes. Adaptation: muck soils of New York, and adjacent states. USDA Circ. 881. 1951.

No. 660 - Breeder and vendor: Dessert Seed Co., El Centro, Calif. Parentage: selection from Great Lakes. Characteristics: very uniform, large head size, dark green foliage, good germination of seeds under warm climatic conditions. Similar: Great Lakes. 1957.

North Pole - Vendor: Cook's Garden. Characteristics: pale green, very cold hardy, adapted for overwintering under cover; ideal size at onset of winter is 4 to 6 inches across. Similar: Arctic King.

Oasis (XP 705) - Breeder and vendor: Asgrow Seed Co. Characteristics: crisphead of Mesa 659 type. Resistance: tolerance to tipburn. Adaptation: Arizona, California, and the eastern United States. 1972.

Optima - Breeder and vendor: Vilmorin, S.A. Characteristics: 52 days to maturity, dark green Nancy type, thick leaves, larger and heavier. Resistant: tipburn and bottom rot. 1995.

Oroverde Great Lakes - Breeder: Waldo Rohnert Co., Gilroy, Calf. Vendor: Aggler and Musser Seed Co. Parentage: single-plant selection Regular Great Lakes. Characteristics: holds dark green color through maturity, sweeter taste than regular Great Lakes. Similar: Great Lakes. Adaptation: western lettuce areas. 1954.

Oswego (76-4) - Breeder: Cornell Univ., Ithaca, N.Y. Characteristics: slower seedstalk development than 456. Similar: Cornell 456. Veg. Crops Mimeo VC-122 June, 1964.

Outback (144 725) - Breeder: Tom McBride. Vendor: Seminis Vegetable Seeds-Asgrow. Parentage: open pollinated. Characteristics: taller Romaine that carries weight well; color and texture of leaf is very similar to current market standards. Resistance: CRR. Similar: Green Towers. Adaptation: coastal California and desert southwest U.S. 1999.

Pace Setter (SSC 30415) - Breeder and vendor: Shamrock Seed Co. Characteristics: iceberg type with excellent uniformity; produces large, firm heads, rounded shape, smooth, flat butts; medium green heads; slightly blistered, crisp leaves; adapted for summer harvest; high tipburn tolerance. Similar: Salinas. Adaptation: California coastal area. 2001.

Pacific - Breeder: E.J. Ryder and Bert J. Robinson. Vendor: USDA, ARS; Salinas, California. Characteristics: crisphead type, dull, medium-dark green leaves, leaf margins similar to Salinas, interior creamy yellow, heads firm to hard at maturity and rounded, well-covered, occasionally spiraled, butt is flat with broad-based overlapping leaves and large core, ribs less flat than Salinas, black seed. Resistance: big vein, downy mildew pathotype 1; susceptible to downy mildew pathotypes 2 and 3, lettuce mosaic and rib discoloration associated with high temperatures. Similar: Salinas. 1981.

Pacific Lakes - Breeder and vendor: Ferry-Morse Seed Co., Mountain View, Calif. Parentage: selection from Reg. Great Lakes. Characteristics: earliness; firm large heads. Resistance: tipburn. Similar: Great Lakes. Adaptation: general use. 1954.

Paleto - Breeder and vendor: Nunhems. Characteristics: dark green, well closed base. Resistance: bolting. 1992.

Palmaro - Breeder and vendor: Nunhems. Characteristics: autumn and winter production. Resistance: mildew, tolerant to lettuce mosaic virus. 1992.

Palmetto - Breeder: Select Seed of California. Vendor: Petoseed. Characteristics: crisphead type, large frame and head, thick dark green leaves, black seed. Resistance: pink rib,

bolting, tipburn, downy mildew California pathovar 3; tolerance to heat, frost. Similar: Vanguard. 1985.

Panther (GX 814) - Breeder: Jodi Grassen. Vendor: Seminis-Genecorp. Parentage: open pollinated. Characteristics: Romaine with medium to dark green colored leaves, smooth leaf surface, tall plant, short core. Resistance: corky root. Similar: Green Towers. Adaptation: west coast and southwestern U.S. 1999.

Paradise - Breeder and vendor: Sakata. Characteristics: crisphead type. Similar: Salinas 88. 1994.

Parris Island COS - Breeder: Clemson College Truck Expt. Sta., Charleston, S.C. Vendor: Ferry-Morse Seed Co. Parentage: PI 120965 x Dark Green. Characteristics: tall, dark green. Resistance: mosaic. Similar: Dark Green COS. Southern Seedsman. 1951.

Parthenon (SSC 1315) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: very uniform and sure heading cultivar similar in production to Winterhaven; plant frame is large and offers good frost protection; produces large, heavy heads with a slightly savoyed leaf texture; heads have a nice, smooth shape and good color. Resistance: big vein. Similar: Colossus. Adaptation: desert Southwest (USA) and winter production. 2004.

Passport (174261) - Breeder and vendor: Seminis Vegetable Seeds. Parentage: open pollinated. Characteristics: dark green mini sized romaine for open field cultivation; suitable for autumn, winter and spring production; good tipburn tolerance. Similar: Pinokkio. Adaptation: NAFTA. 2004.

Patriot - Breeder and vendor: Asgrow. Characteristics: crisphead type, medium size for summer harvest in coastal California, medium dark green, frame larger than Warrior. Resistance: downy mildew pathotypes 1, 2, 3. Similar: Salinas, Warrior. 1991.

Pennlake - Breeder: Pennsylvania Agr. Expt. Sta., State College, and Pieters-Wheeler Seed Co., Gilroy, Calif. Vendor: Pieters-Wheeler Seed Co. Parentage: Great Lakes x Imperial 847. Characteristics: uniformity of heading, solidity, earliness. Resistance: some to tipburn, tolerant to heat. Similar: Great Lakes. Adaptation: eastern United States. Pa. Bul. 502, Suppl. 2. 1949.

Phoenix - Breeder and vendor: Dessert Seed Co., El Centro, Calif. Parentage: selection from Great Lakes. Characteristics: good head formation, dark green foliage, very smooth base, large head. Similar: Great Lakes. 1957.

Pinecrest (29709) - Breeder and vendor: Enza Zaden. Parentage: open pollinated. Adaptation: North America.

Plato - Breeder and vendor: Petoseed. Characteristics: cos type, medium green head, savoyed leaves, crisp, upright, impeccable quality. Resistance: bolting, lettuce mosaic virus, tipburn. 1989.

Pliant - Breeder and vendor: Orsetti Seed Co. Parentage: open pollinated.

Premier Great Lakes - Breeder: Pennsylvania Agr. Expt. Sta., State College, and the Pieters-Wheeler Seed Co., Gilroy, Calif. Vendor: Pieters-Wheeler Seed Co. Parentage: line selection of Great Lakes. Characteristics: large, solid, head 10 days earlier than standard Great Lakes, uniform in heading. Resistance: tipburn, similar to that of Great Lakes. Similar: Great Lakes. Adaptation: eastern United States. Pa. Bul. 502, Suppl. 2. 1949.

Presidio - Breeder and vendor: Asgrow. Characteristics: cos type, dark green blistered leaves, plant upright and compact with open heart, thick leaves. Resistance: corky root rot, lettuce mosaic virus, Bidens mottle virus, bolting. Similar: Parris Island. Adaptation: most lettuce growing areas. 1991.

Primaverde Great Lakes - Breeder and vendor: Waldo Rohnert Co., Gilroy, Calif. Parentage: Great Lakes. Characteristics: tolerant to tipburn, does not get large and puffy under high temperatures. Similar: Great Lakes. Adaptation: early fall and late spring desert areas and eastern areas. 1957.

Prime Time - Breeder and vendor: Asgrow. Characteristics: crisphead type, large frame and head, thick dark green leaves, large butt, medium ribs, black seed. Resistance: pink rib, tipburn, bolting, tolerance to frost. Similar: Vanguard. 1993.

Prizeleaf - Vendor: Burpee. Characteristics: leaf type.

Progress - Breeder: New Jersey Agr. Expt. Sta., New Brunswick, and Bureau of Plant Industry Station, USDA, Beltsville, Md. Parentage: selection from cross of Imperial 44 with an unnamed hybrid. Characteristics: white seed, early, dark green, thick leaved, heavily savoyed, crisphead lettuce of good flavor and high quality. Resistance: tipburn. Adaptation: general. Southern Seedsman Reprint.

Pybas No. 251 M.T. - Breeder and vendor: Pybas Vegetable. Characteristics: crisphead type, large frame and head, medium leaf thickness, medium to dark green color, medium to large butt, slightly ribby, black seed. Resistance: bolting, pink rib, tipburn, big vein, downy mildew California pathovar 3; tolerance to heat and frost. Similar: Vanguard. 1992.

Quechan - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: crisphead type; 60-65 day maturity; produces large, medium green heads; large frame, heads larger than Empire, smooth ribs; uniform plant type and tolerance to tipburn and bolting. Resistance: bolting, pink rib, tipburn; tolerance to heat and frost. Similar: Acacia, Empire. Adaptation: Arizona, California. 1996.

Ramona - Breeder: University California, Davis Vendor: USDA, and University California, Davis. Characteristics: crisphead type, firm, butt appearance good, seedstalk low, white seed. Resistance: tipburn symptoms vary between none, less than a trace. 1978.

Ramses (LM 9615) - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: green butter lettuce; 50-65 day maturity; produces medium heads, leaves are thick, medium green in color and smooth. Resistance: downy mildew; tipburn. Similar: Boston Bibb. Adaptation: can be cultivated in U.S. if sown in proper season.

Rave - Breeder and vendor: Johnny's Selected Seeds. Parentage: open pollinated. 2000.

Red Bull (PS 06515636) - Breeder and vendor: Seminis Vegetable Seeds. Parentage: open pollinated. Characteristics: very large looseleaf type; dark red with smooth leaf margin and smooth leaf surface; wide adaptation; tip burn and bolting resistant. Similar: Red Chalte, New Red. Adaptation: NAFTA, southwest coastal U.S. PVP application #200500042. 2005.

Red Butterworth (169398) - Breeder: Jim Waltrip. Vendor: Seminis-Petoseed. Parentage: open pollinated. Characteristics: suitable for spring, summer, and autumn harvest, resists bolting when stressed, heads are heavy, firm, green color, leaves are blistered. Adaptation: wholesale and home garden. 2000.

Red Coach 74 - Breeder and vendor: Quali-Sel, Inc. Characteristics: open pollinated; large frame and head size. Resistance: none claimed. Similar: Vanguard. Adaptation: midwinter and early spring harvest for Arizona and California. PVP Certificate No. 7400010. 1979.

Red Coach 74A - Breeder and vendor: Quali-Sel, Inc. Characteristics: open pollinated; bright green frame and wrapper leaves. Resistance: none claimed. Similar: Vanguard. Adaptation: late fall plantings for Arizona and California. PVP Certificate No. 7400024. 1979.

Red Dawn - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: red oakleaf lettuce; 45-65 day maturity; medium to large heads; red leaf color; excellent shelf life. Resistance: downy mildew. Similar: Red Salad Bowl. Adaptation: can be cultivated in U.S. if sown in proper season.

Red Embers - Vendor: Brinker. Characteristics: cos type, deep red color. Resistance: bolting. Similar: Deep Red. 1988.

Red Gem (LM 4965) - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: red Lolla Rossa lettuce; 45-65 day maturity; medium sized heads; deep red color; very frilled leaves; synonym is Impuls. Similar: Lolla Rossa. Adaptation: can be cultivated in U.S. if sown in proper season. 1997.

Red Great Lakes - Breeder and vendor: Dessert Seed Co., El Centro, Calif. Parentage: Great Lakes. Characteristics: outer leaves turn red, firm head, good quality. Similar: Great Lakes. Adaptation: southwest U.S. 1959.

Red Green - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: red Lolla Rossa lettuce; 45-65 day maturity; medium sized heads; deep red color; very frilled leaves. Similar: Lollo Rossa. Adaptation: can be cultivated in U.S. if sown in proper season. 1997.

Red Grenoble - Breeder: Vilmorin. Vendor: Cook's Garden. Characteristics: shiny, wine red leaves, vigorous grower, resistant to adverse conditions, can be cut young or left to head up; Batavia type. 1932.

Red Romaine (COS) - Breeder and vendor: Dessert Seed Co., El Centro, Calif. Parentage: selection out of an introduction from Africa. Characteristics: very tender, sun red, outer

leaves. 1950.

Red Rustler - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: red loose-leaf lettuce, 45-65 day maturity, medium large heads; deep red leaf color; very frilled leaves. Similar: New Red Fire. Adaptation: can be cultivated in U.S. if sown in proper season.

Red Sails - Breeder and vendor: Seminis Vegetable Seeds-Petoseed. Characteristics: 45 days to maturity, fast growing, heat-tolerant and slow to bolt, open heads up to a foot across, deepens in color as they mature, bronze-red leaves, holds color and sweetness in hot weather. 1986.

Red Salad Bowl - Breeder and vendor: Dessert Seed Co., El Centro, Calif. Parentage: out of Saladbowl. Similar: Saladbowl, but red color. Adaptation: U.S.

Red Salad Trim (COS) - Breeder and vendor: Dessert Seed Co., El Centro, Calif. Parentage: out of Red Romaine. Characteristics: very sow to bolt, very sweet, dark red, can be used for ornamental plant, also good to eat. Adaptation: United States.

Red Sceptre - Breeder and vendor: Asgrow. Characteristics: leaf type, bright red tinge to outer leaf area. Resistance: bolting. Similar: Prizehead. 1987.

Red Velvet - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: red butter lettuce; 45-65 day maturity; medium heads; deep red outer leaf color; blistered leaf texture; yellow green inner leaves. Similar: Merveille de 4 Saisons, Salinas. Adaptation: can be cultivated in U.S. if sown in proper season. 1997.

Red Vogue - Breeder and vendor: Sakata. Characteristics: butterhead type, red. Similar: Butter Red Leaf. 1990.

Redhead (XP 438) - Breeder and vendor: Asgrow Seed Co. Characteristics: open pollinated; leaf lettuce with very intense uniform red color on the outer half of leaf. Resistance: good level of heat tolerance. Similar: Prizehead and Ruby. PVP Certificate No. 9000095. 1981.

Redina - Breeder and vendor: Enza Zaden. Characteristics: reddest cultivar of Grand Rapids type, color is intense candy red, green butt, pliable, non-brittle ribs, slow to tipburn; red leaf. 1995.

Redleaf (XP 438) - Breeder and vendor: Asgrow Seed Co. Characteristics: open pollinated; red color is more intense and less dispersed compared to Prizehead with some red color extending into the surface layers of the petioles and stems. Resistance: none claimed. Similar: Prizehead. Adaptation: wide. PVP application pending. 1980.

Redondo - Breeder and vendor: Synergene. Characteristics: crisphead type, moderate, large frame and head, excellent size and texture for wrap or bulk harvest, thick crisp dark glossy green wide wrapper leaves, smooth ribs, small core, white seed. Resistance: bolting, tipburn; tolerance to heat. Similar: Vanguard-Empire. 1992.

Regiment (SSC 30391) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Resistance: downy mildew. Similar: Lollo Rossa. Adaptation: all lettuce production regions. 1999.

Reine des Glaces - Breeder: Vilmorin. Vendor: Cook's Garden. Characteristics: deeply notched leaves and a convoluted head of frosty green; best results from spring plantings; Batavia type. 1883.

Remington (SSC 30474) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Similar: Pam's Island Cos. Adaptation: all major lettuce growing regions. 2001.

Rhapsody (SSC 30390) - Breeder and vendor: Shamrock Seed Co. Resistance: downy mildew. Similar: Baja. Adaptation: California, Arizona, and other butter lettuce production regions.

Rico - Breeder and vendor: Synergene. Characteristics: crisphead type, large to very large frame, large head, thick crisp dark green leaves, medium large butt, smooth ribs, white seed, early plantings, excess fertilizer and water may promote extra large, wild, poorly headed plants, holds well under very cold, wet conditions and repeated frosts. Resistance: bolting, downy mildew California pathovar 1, frost; tolerance to heat. 1989.

Ridgemark II (GX 607) - Breeder: Debbie Mitchell. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: F1 hybrid. Characteristics: grayish green color, good bolting tolerance, flat butt, smooth petioles. Similar: Raider. Adaptation: coastal areas of California. 1997.

Rio Verde - Breeder and vendor: Shamrock. Characteristics: crisphead type, medium to large frame and head, leaf color darker than Empire, smooth ribs and butt. 1995.

Rita - Breeder: University California, Davis. Vendor: USDA, and University California, Davis. Characteristics: crisphead type, medium green, leaf margins of wrapper leaves have a smooth cabbage-like appearance, head size is 24, solid, good butt, smooth, large cores, low seedstalk. Resistance: possibly tipburn, probably contains PI 91532, downy mildew resistant Lactuca serriola introduction from Russia. 1978.

Rolina - Breeder and vendor: Enza Zaden.

Romulus - Breeder and vendor: Petoseed. Characteristics: cos type, medium green color, slightly savoyed leaves, good yields. Resistance: lettuce mosaic virus, corky root rot. 1989.

Rosa - Breeder and vendor: Arco Seed Co. Characteristics: open pollinated; red head lettuce. 1981.

Rosalita - Vendor: Johnny's. Characteristics: cos type, deep red on inner exposed leaf surfaces, green outer base, early maturity, spring, fall, and winter crops. Similar: Rouge D Hiver.

Rossada (21504) - Breeder and vendor: Enza Zaden. Parentage: open pollinated. Adaptation: North America. 1997.

Rosseto (G x 915) - Breeder: Debbie Mitchell. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: medium to dark red Lolla Rossa. Similar: no cultivar like it. Adaptation: U.S.

Rougette du Midi - Breeder: Vilmorin. Vendor: Cook's Garden. Characteristics: also known as Red Montpelier; red butterhead type for baby lettuce or off-season covered crops; not recommended for summer. 1842.

Royal Green - Breeder and vendor: Royal Sluis B.V. Characteristics: 45 days to maturity, darker green version of Grand Rapids, very uniform, green leaf. Resistance: tipburn. 1988.

Royal Oak Leaf - Breeder and vendor: W. Atlee Burpee Co. Parentage: Oak Leaf x Burpee Bibb. Characteristics: open pollinated; larger rosette with darker green leaf color than Oak Leaf. Resistance: heat and tipburn. Similar: Oak Leaf. Adaptation: wide. PVP Certificate No. 7800009. 1978.

Royal Red - Breeder and Vendor: Royal Sluis B.V. Characteristics: 45 days to maturity, deep red version of Grand Rapids, larger frames than Red Rapids but not as large as Red Sails, red leaf. Resistance: tipburn. 1988.

RS 0254 - Breeder: Royal Sluis America. Vendor: Petoseed. Characteristics: crisphead type, very large frame and head, thick dark green leaves, smooth ribs, black seed. Resistance: pink rib, tipburn, big vein, downy mildew California pathovar 3, frost; tolerance to heat. Similar: Vanguard. 1994.

RS 0319 - Breeder: Royal Sluis America. Vendor: Petoseed. Characteristics: crisphead type, large frame and head, thick dark green leaves, smooth ribs, black seed. Resistance: bolting, pink rib, tipburn, big vein, downy mildew California pathovar 3, frost; tolerance to heat. Similar: Vanguard. 1994.

Rubra - Breeder and vendor: Arco Seed Co. Characteristics: Red Cos Lettuce. 1981.

Ruby - Breeder: USDA, Beltsville, Md. Parentage: Prizehead x complex hybrid. Similar: Prizehead. Characteristics: intense red color that holds under conditions where color fades in other red cultivars. Adaptation: wide. 1957.

Sahara (GS 06511294) - Breeder and vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: Iceberg type, suitable for high temperature regions, large frame, medium green with an outstanding bolting tolerance; best early seson planting in Huron on August 13-25, Yuma on September 5 to 20, spring or summer market on June 1 to September 1; Chile on December 1 to January 30. Similar: Sun Devil, Beacon, Light House, Valley Green. Adaptation: Desert Southwest U.S. and Huron, California. 2002.

Saguaro - Breeder and vendor: Harris Moran. Characteristics: cos type, very large frame. Resistance: lettuce mosaic virus, corky root rot. Similar: Parris Island Cos.

Salad Bibb (HXP 3550) - Breeder: Dr. Hasib S. Humaydan. Harris Moran Seed Co. Characteristics: open pollinated; butterhead type lettuce with multiple disease resistance. Resistance: lettuce mosaic virus, broad bean wilt virus and downy mildew. Similar: Summer

Bibb. Adaptation: northeast, midwest and southern United States. PVP No. 8500060. 1985.

Salad Bowl - Breeder: Plant Industry Sta., USDA, Beltsville, Md. Parentage: a complex hybrid. Similar: Oak Leaf. Characteristics: heavily lobed leaves, very slow bolting, high quality, high in vitamins A and C. Resistance: tipburn and high temperatures.

Salinas - Breeder: E.J. Ryder. Vendor: USDA, ARS; Salinas, California. Characteristics: crisphead type, dull green outer leaves, leaf margins scalloped or wavy, texture soft, creamy interior, firm to hard at maturity, spherical, slightly bald on top, adequately covered, bottom slightly flattened with flat ribs, leaves broad at base, core diameter medium to large, black seed. Resistance: tipburn; susceptible to downy mildew V8, lettuce mosaic, western yellows, and turnip mosaic viruses; Sclerotinia; sensitive to ethylene, russet spotting and pink rib. 1975.

Salinas 88 - Breeder: E.J. Ryder. Vendor: USDA, ARS; Salinas, California. Characteristics: crisphead type, dull, dark green outer leaves, heads firm to hard at maturity and well rounded, green pigmentation extends close to core, interior creamy yellow, flat butt and ribs, large core, leaf surface slightly crinkled, outer leaves are broader than long, black seed. Resistance: tipburn, tolerance to lettuce mosaic virus; susceptible to big vein and corky root. Similar: Salinas. 1988.

Salmar (E8248) - Breeder: Ferry-Morse Seed Co. Characteristics: open pollinated; very smooth butt. Resistance: downy mildew. Similar: Salinas. Adaptation: spring harvest on the central coast of California. PVP application pending. 1981.

Salverde - Breeder: Dr. G. Emery. Vendor: Ferry-Morse. Characteristics: crisphead type, slightly larger head, tighter wrappers and more serrated leaf margins than Salinas. Resistance: downy mildew. Similar: Salinas. 1988.

Sangria - Vendor: Vilmorin. Characteristics: butterhead type, first butterhead resistant to bolting, cropping spring, summer, and autumn, intensive red color in spring, brownish red in summer, heavy heads 450-650 g well shaped, no off shoots. Resistance: bolting, Bremia R11, lettuce mosaic virus. Similar: Merveille des 4 Saisons. 1987.

Santa Fe - Breeder and vendor: W. Atlee Burpee Co.

Santans (SSC 30473) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Similar: Vanguard. Adaptation: Yuma, Arizona and Imperial Valley. 2001.

Sea Green - Breeder: E.J. Ryder. Vendor: USDA, ARS; Salinas, California. Characteristics: crisphead type, dull green leaves, interior is creamy, heads firm and well formed, core diameter medium to large, low to medium stem. Resistance: big vein; susceptible to downy mildew, lettuce mosaic and Sclerotinia. 1981.

Sedona - Breeder: Royal Sluis America. Vendor: Petoseed. Characteristics: crisphead type, large frame and head, thick dark green leaves, smooth ribs, black seed. Resistance: bolting, pink rib, tipburn; tolerance to heat and frost; susceptible to big vein; downy mildew California pathovar 3. Similar: Vanguard. 1991.

Sentry (SSC 30399) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Resistance: downy mildew. Similar: Lollo Rossa. Adaptation: all lettuce Production regions. 1999.

Sequoia - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: Crisphead type; maturity is 90-110 days; large, dark green heads; extremely good in cool weather. Similar: Pybas 251. Adaptation: California central coast. 1996.

Shilo - Breeder and vendor: Asgrow. Characteristics: crisphead type, mid-winter desert conditions in California and Arizona, large head and frame with excellent dark green color. Similar: Kofa. 1993.

Shogun - Breeder: Haruo Sakamoto. Vendor: Ferry-Morse. Characteristics: crisphead type, slightly gray-green color, soft leaves, good wrappers. Resistance: downy mildew, common strain). Similar: Calmar. 1983.

Sure Shot (PX 06512772) - Breeder and vendor: Seminis Vegetable Seeds-Petoseed. Parentage: open pollinated. Characteristics: very attractive head, minimal ribbyness, medium dark exterior cover and wrap with a short core; uniform round shape and flat bottom makes it suitable for fresh, cello, or process type; dense interior with minimal space between internodes makes this cultivar heavy and firm. Resistance: downy mildew, big vein, corky root rot. Similar: Trojan, Jupiter, Big Ben, Pybas 251. Adaptation: Southwest Coastal California. 2002.

Sicily (GX 923) - Breeder and vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: spring mix, red frilly type, triple red Rossa type with excellent texture and very frilly leaf margin. Similar: Lolla Rossa. Adaptation: California coast and cooler climate planting conditions. 2002.

Sierra - Vendor: Vilmorin. Characteristics: Batavia type, red, cropping in spring, summer and autumn, upright form and large size. Resistance: bolting, Bremia R11, tipburn. Similar: Rossia, Canasta. 1988.

Signal - Breeder: T.W. Whitaker and J.D. McCreight. Vendor: USDA, Agric. Res. Stn.; Salinas, California. Characteristics: cos type, leaves upright, cap over the head rather than remaining open, head tapered slightly towards the butt, results in a "blocky" appearance, sweet flavor, firm, uniform. 1980.

Simpson Elite - Breeder and vendor: Petoseed. Characteristics: leaf type, bright yellow green looseleaf, excellent uniformity and leaf quality. Resistance: bolting. Similar: Black Seeded Simpson. 1993.

Simpson Elite - Breeder and vendor: Twilley Seeds. Characteristics: 45 days to maturity, slow bolting, medium light green, leaves are attractive, broad and crumpled with curled outer leaf margins. 1998.

Siskiyou - Breeder: Doug Sousa. Vendor: Seminis Vegetable Seeds-Petoseed. Parentage: open pollinated. Characteristics: medium green, blistered leaf, Romaine, open plant habit, good internal blanching. Resistance: corky root, LMV. Similar: Green Towers. Adaptation:

coastal California. 1999.

Skyline - Breeder and vendor: Asgrow. Characteristics: crisphead type. Resistance: bolting. Similar: Ithaca 989. Adaptation: northeast U.S, Canada. 1993.

Sky/Line - Breeder and vendor: Orsetti Seed Co. Adaptation: U.S. 2005.

Slider (G x 818) - Breeder: Jodi Grossen. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: medium sized Romaine, narrow base and wide mid-section, well suited for hearts due to torpedo shape. Resistance: corky root. Adaptation: Florida. 1999.

Slobolt - Breeder: Bureau Plant Industry Station, USDA, Beltsville, Md. Parentage: Giant Summer x Grand Rapids. Characteristics: slow bolting Grand Rapids type. Resistance: high temperatures and tipburn. Adaptation: wide. Southern Seedsman. August, 1945.

Snappy (G x 817) - Breeder: Jodi Grossen. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: medium sized Romaine, very bright green leaves, late closing. Resistance: corky root. Similar: no cultivar like it. Adaptation: Florida. 1999.

Sniper (PS 3879) - Breeder: Doug Sousa. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: Large heading dark green crisphead lettuce, large frame to provide good head protection. Resistance: corky root. Similar: Sharp Shooter. Adaptation: Coastal California. 1999.

Snowbird - Breeder and vendor: Ferry-Morse. Characteristics: crisphead type, large frame and head, medium thick dark olive green leaves, large butt, slightly ribby, black seed. Resistance: slight bolting, pink rib, tipburn, big vein, downy mildew California pathovar 3; tolerance to frost. Similar: Vanguard. 1988.

Sonora - Breeder and vendor: Harris Moran. Characteristics: crisphead type, very large frame, dark green glossy leaves. Resistance: bolting, lettuce mosaic virus, downy mildew pathotype 2. Similar: Vanguard.

Spartan Lakes - Breeder: Michigan St. Agric. Exp. Sta. Vendor: Michigan Agric. Exp. Stn., Michigan State University, East Lansing. Characteristics: crisphead type, early maturity, small size, dark green color, strong butt structure. Resistance: bolting. Adaptation: Michigan mucklands. 1968.

Sphinx - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: 40-45 day maturity; medium heads; light to medium green color; smooth leaf texture. Resistance: downy mildew; tipburn; bolting. Similar: Boston Bibb. Adaptation: can be cultivated in U.S. if sown in proper season. 1999.

Squadron (SSC 30543) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: baby leaf type that produces thick textured leaves with frilly leaf margins and a dark, lime green color; suitable as a replacement for Tango, particularly under downy mildew incidence; also has applications as a full grown product in certain markets.

Resistance: downy mildew resistance (pathotypes I, IIA, IIB, III, IV and V). Similar: Waldmanns Green. Adaptation: U.S. lettuce production regions. 2002.

Steinbeck (GS 632) - Breeder: Lisa Muss. Vendor: Seminis-Genecorp. Parentage: open pollinated. Characteristics: large iceberg, medium green color, excellent texture, main growing season. Resistance: corky root. Similar: Sharpshooter. Adaptation: Salinas Valley, California February to June plantings. 2000.

Sudia - Vendor: Vilmorin. Characteristics: semi-early cropping in spring, summer, and autumn, dark green matte foliage very thick and dimpled, semi-long heads 400 g. Similar: Sucrine, Sudel, Bella. 1984.

Sumatra - Breeder and vendor: Asgrow. Characteristics: cos type, light green color, very large, well-closed heads, early maturity. Resistance: bolting, cold tolerant. Similar: Bionda Collosseo. 1990.

Sumi - Breeder: Quali-Sel, Inc. Characteristics: open pollinated; very short core length. Resistance: none claimed. Similar: Amaral 400. Adaptation: Yuma, Arizona, San Joaquin and Imperial valleys, Calif. PVP Certificate No. 8000134. 1981.

Summer Bibb (B52) - Breeder: Cornell Univ., Ithaca, N.Y. Characteristics: does not bolt to seed as rapidly as Bibb. Similar: Bibb. Veg. Crops Mimeo VC-123, June, 1964.

Summer Queen (XP 514) - Breeder and vendor: Asgrow Seed Co. Characteristics: open pollinated; slow bolting butterhead for summer growing conditions. Resistance: none claimed. Similar: Dark Green Boston. Adaptation: United States and Canada. 1978.

Summertime - Breeder: J.R. Baggett. Vendor: Oregon Agric. Exp. Sta. Characteristics: crisphead type, slow to become bitter in summer. Resistance: bolting, tipburn, brown rib. Similar: Ithaca. 1989.

Sundowner - Breeder and vendor: Asgrow. Characteristics: crisphead type, dark green color, excellent heading ability, smooth butt. Resistance: bolting. Similar: Empire. 1993.

Sunset - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: Red Oak leaf lettuce; 45-65 day maturity; intense red leaf color; compact heads. Resistance: downy mildew. Similar: Red Salad Bowl. Adaptation: can be cultivated in U.S. if sown in proper season. 1996.

Super 59 - Breeder and vendor: Ferry-Morse. Characteristics: crisphead type, medium frame, head, and ribs, medium thick dark forest green leaves, large butt, white seed. Resistance: bolting, pink rib, tipburn; tolerance to heat and frost. Similar: Great Lakes 659. 1972.

Superbb - Breeder and vendor: Michigan Agr. Expt. Sta. Parentage: Noran x Bibb. Characteristics: open pollinated; tolerant of low-light intensity; adapted to a wide range of day lengths, earlier than Bibb, high quality low shattering leaves at harvest. Resistance: certain strains of downy mildew. Adaptation: greenhouse and early and late crops in the field. 1980.

Sweet Gem - Breeder and vendor: Asgrow. Characteristics: dark green color, compact plant, excellent taste. Resistance: bolting. Similar: Little Gem. 1994.

Sweetheart - Breeder and vendor: Waldo Rohnert Co., Gilroy, Calif. Parentage: a butter head of White Boston type. Similar: White Boston. Characteristics: nonbolting, tolerant to tipburn. Adaptation: wide. 1957.

Sweetie B.S. - Breeder and vendor: ARCO/Dessert Seed Co. Characteristics: semi-Cos type leaf lettuce. 1981.

TAMU Valrio - Breeder: T.W. Whitaker and P.W. Leeper. Vendor: Texas Agric. Exp. Stn., Texas A and M University Weslaco, Texas. Characteristics: crisphead type, well colored, leaves have straight flat ribs, flat butt. Resistance: downy mildew; tolerance to cold. 1968.

TAMU Valtemp - Breeder: T.W. Whitaker and P.W. Leeper. Vendor: Texas Agric. Exp. Stn., Texas A and M University Weslaco, Texas. Characteristics: crisphead type, adapted to early season planting in high temperatures, exceptionally good head color and quality, uniform head size and maturity, dark green, shorter stems, heads closer to ground, flat butt, good color, ribs straight and flat. Resistance: bolting, downy mildew; tolerance to cold. 1968.

Target - Breeder and vendor: Petoseed. Characteristics: crisphead type, excellent color, very firm medium green head, smooth butt. Resistance: downy mildew California pathotypes 1, 2. Similar: Salinas. 1990.

Tarragona (G x 920) - Breeder: Frank Casellas. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: Tango type, lobed leaf; color darker than Tango, leafy thicker than Tango. Similar: Tango. Adaptation: all areas-specialty. 1998.

Tempe (XP 48) - Breeder and vendor: Asgrow Seed Co. Characteristics: crisphead type. Resistance: tolerance to tipburn. Similar: Golden State D. Adaptation: spring production in Arizona and coastal California and early winter harvest in Imperial Valley of California. 1972.

Tendercrisp - Breeder and vendor: Keystone Seed Co. Characteristics: Bibb type, with glossier appearance, crisp, high quality. Similar: Bibb types. Adaptation: same as Bibb. Keystone Catalog. 1969.

Tendergreen - Breeder: Michigan State Univ., East Lansing. Vendor: Grand Rapids Growers. Parentage: Grand Rapids x Bibb. Similar: Bibb. Characteristics: stalk large and upright, foliage dark green, slightly curled. Adaptation: greenhouse in north in winter. 1955.

Terrapin (G x 816) - Breeder: Jodi Grossen. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: very large, bright green color, Romaine type with smooth leaf surface, slow bolting, slow closing, heavy, excellent for cartons and processing. Resistance: corky root. Similar: no cultivar like it. Adaptation: Florida. 1999.

Thompson - Breeder: E.J. Ryder. Vendor: USDA, ARS; Salinas, California. Characteristics: crisphead type, medium dark green outer leaves, leaf margins dentate and texture is crisp, interior light cream, nearly white, heads firm to hard at maturity and well-covered, occasionally spiraled, butt is usually flat, occasionally rounded, leaves broad at base, ribs

usually flat occasionally raised, core medium to large, white seed. Resistance: big vein; susceptible to downy mildew, tipburn, lettuce mosaic and Sclerotinia. 1981.

Tiara - Breeder and vendor: Asgrow. Characteristics: leaf type, blistered, frilled, looseleaf, dark green leaves, erect. Resistance: tipburn, slow bolting. Similar: Waldmanns Green. 1989.

Tiber - Breeder: USDA, Agric. Res. Stn., Salinas, California. Vendor: USDA, Agric. Res. Stn. Characteristics: crisphead type, large to very large size, head firm to hard, dull, dark green outer leaves, leaf margins slightly incised, indented and undulate, soft texture, leaf surface moderate crinkle, interior creamy yellow, well rounded, flat butt and ribs, large core, low to medium stem, black seed. Resistance: tipburn.

Tigris - Breeder: Leen de Mos Vegetable Seeds. Vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: red loose leaf lettuce; 45-65 day maturity; medium large heads; intense red leaf color; broad leaves with blistered texture; upright plant habit. Resistance: tipburn. Similar: New Red Fire. Adaptation: can be cultivated in U.S. if sown in proper season. 1997.

Time - Breeder and vendor: Asgrow. Characteristics: crisphead type, dark green, blistered leaves, medium size frame, good heading ability, flat butt provides excellent boxy appearance. Similar: Honcho 2. 1993.

Top Gun - Breeder and vendor: Asgrow. Characteristics: crisphead type, medium to large, spring harvest in California coastal areas, medium to dark green color. Resistance: downy mildew pathotypes 1, 2, 2. Similar: Salinas. 1991.

Torino (G x 904) - Breeder: Debbie Mitchell. Vendor: Seminis Vegetable Seeds-Genecorp. Parentage: open pollinated. Characteristics: Red Oak leaf type with longer leaf, deeper lobes than Valencia. Similar: Valencia. Adaptation: U.S. 1997.

Tortuga - Breeder and vendor: Asgrow. Characteristics: cos type, medium green, shiny color, heads upright nicely formed, well closed. 1990.

Tourist (164553) - Breeder and vendor: Seminis Vegetable Seeds. Parentage: open pollinated. Characteristics: dark green romaine type, leaves large size, shiny color, slightly blistered (savoyed); compact plant, cold tolerant, good weight, bolting tolerant. Resistance: downy mildew (BL 01-16, 19, 21, 23). Similar: Bacio, Remus. Adaptation: NAFTA. 2004.

Tracer - Breeder: Doug Sousa. Vendor: Seminis Vegetable Seeds-Petoseed. Parentage: open pollinated. Characteristics: Salinas type crisphead, high corky root tolerance, good color, large heads; poor head formation under warm conditions. Resistance: tolerant to corky root. Similar: Cowboy, Wrangler. Adaptation: coastal California. 1997.

Trendsetter - Breeder and vendor: Asgrow. Characteristics: crisphead type for late Empire to early Merit planting slot, dark green, smooth butt appearance. Similar: Empire. 1993.

Tres Equis - Breeder and vendor: Synergene. Characteristics: crisphead type, large frame, moderate large head, thick moderately crisp dark dull to glossy green leaves, small butt,

small core, smooth ribs, dark brown seed. Resistance: bolting, tipburn, pink rib; tolerance to heat. Similar: Vanguard. 1992.

Trinidad - Breeder: K. Bixby. Vendor: Asgrow. Characteristics: butterhead type, blonde leaves, medium size heads. Resistance: downy mildew. Similar: Joric Le. Adaptation: Northern Mediterranean region. 1987.

Triple Threat (PS 06516604) - Breeder and vendor: Seminis Vegetable Seeds. Parentage: open pollinated. Characteristics: Romaine, Paris Island Cos type; medium green color; slightly closed, slightly blistered large leaf; very tolerant to tip burn and bolting; can be used by processor, fresh market and hearts segment; suggested sow dates: March 1 to September 15. Similar: Green Towers, Hearts Delight. Adaptation: Southwest Coastal U.S., NAFTA. 2004.

Triton (HMX 7555) - Breeder: Philip Sarreal Vendor: Harris Moran Seed Co. Parentage: open pollinated. Characteristic: large size, excellent vigor, dark green, slightly savoyed. Similar: Green Towers. Adaptation: Coastal California, Desert, Southwest, Eastern U.S. 2001.

Tuscany (G x 825) - Breeder: John Purcell. Vendor: Seminis-Genecorp. Parentage: open pollinated. Characteristics: Green Romaine spring mix, dark color, slightly blistered leaves. Similar: Granada. Adaptation: western U.S. 2000.

Two Star - Breeder and vendor: Brinker. Characteristics: leaf type, darker green, more uniform, larger size than Waldmanns Green. Similar; Waldmanns Green.

Ultra Green - Breeder and vendor: Brinker. Characteristics: leaf type, dark green color, uniform. Resistance: tipburn. Similar: Waldmanns Green.

Ultra Red (BOS 9019) - Breeder and vendor: Orsetti Seed Co. Parentage: open pollinated. Characteristics: bright red leaf; bottom side of leaf lighter green; green stem. Similar: Vulcan. Adaptation: widespread in North America. 1997.

Valcos - Breeder: Mr. Paul Leeper. Vendor: Texas Agric. Exp. Stn., Weslaco. Characteristics: cos type, center leaves are bright yellow, dark green outer leaves, heads cap well, compact, straight ribs. Resistance: bolting, tipburn; downy mildew race 5, 6.

Valley Heart (RX 06511508) - Breeder and vendor: Seminis Vegetable Seeds. Parentage: open pollinated. Characteristics: Romaine type; medium green color, slightly blistered and large leaf, semi closed, narrow bottom, tall growth habit; non-glossy leaves, smooth leaf margin; very good cold tolerance combined with good bolting and tip burn tolerance; can be used by processor for fresh and hearts segments. Similar: King Henry, Green Towers. Adaptation: NAFTA, southwest coastal, cool season desert U.S. PVP application #200500041. 2005.

Valmaine (Texas 1087) - Breeder: Texas Agr. Expt. Sta., Weslaco, and USDA, La Jolla, Calif. Parentage: PI 167150 and complex Cos hybrid. Characteristics: uniform, dark green, slightly savoyed, large, early maturing. Resistance: downy mildew. Similar: Parris Island Cos. Adaptation: lower Rio Grande Valley, Texas. Amer. Veg. Grower, Fall 1963.

Valprize - Breeder: Mr. Paul Leeper. Vendor: Texas Agric. Exp. Stn., Weslaco, Texas. Characteristics: butterhead type, dark green color, large head and stem, straight, flat ribs, excellent butt appearance. Resistance: premature bolting, tipburn; downy mildew race 5, 6.

Valta - Breeder: K. Bixby. Vendor: Asgrow. Characteristics: cos type, small, compact. Resistance: lettuce mosaic virus. Similar: Parris Island Cos. 1987.

Valverde - Breeder: USDA La Jolla, Calif. Vendor: USDA and Texas Agr. Exp. Station, College Station, Texas. Parentage: PI from Russia crossed with several Imperial types and Cosberg. Characteristics: dark green, thick wrapper leaves, head firm, well folded, good butt appearance. Resistance: downy mildew. Similar: Great Lakes. Adaptation: lower Rio Grande Valley of Texas. Amer. Vegetable Grower 9. 1959.

Van Mor (Vanguard 551) - Breeder and vendor: Moran Seeds, Inc. Characteristics: open pollinated; Vanguard type; heads uniform medium dark green. Resistance: tolerant to tipburn and dormancy at high temperatures. Similar: Vanguard. Adaptation: desert areas. PVP No. 8100172. 1980.

Vancrisp - Breeder: Quali-Sel. Vendor: Petoseed. Characteristics: crisphead type, large frame and head, thick dark green leaves, smooth ribs, black seed, small core and butt. Resistance: pink rib, tipburn, downy mildew California pathovar 3; tolerance to heat and frost; susceptible to big vein. Similar: Vanguard. 1986.

Vanguard - Breeder: USDA, Beltsville, Md. Parentage: Climax, 5192 (derived from I. virosa x L. sativa), 4157 (original parents Imp. 847, Cosbia, Grand Rapids, and Salamander). Characteristics: dark, dull green color, excellent eating quality, large size, outstanding butts. Adaptation: Salinas-Watsonville district, Calif. 1959.

Vanguard 75 - Breeder: E.J. Ryder. Vendor: USDA, ARS; Salinas, California. Characteristics: crisphead type, large frame leaves tend to enclose the head, large, thick dull green leaves, broad flat butt, occasionally slightly pointed, smooth flat ribs, black seed, used widely for late spring harvest in desert southwest United States since late 1970s; first cultivar with lettuce mosaic virus resistance. Resistance: bolting; tipburn; lettuce mosaic virus; tolerance to heat and frost Similar: Vanguard. 1975.

Vanmax (E 0201) - Breeder and vendor: Ferry-Morse Seed Co. Characteristics: earlier with larger frame and head, more uniform maturity, slightly lighter color than Vanguard. Similar: Vanguard. 1972.

Vega - Breeder: University California, Davis. Vendor: USDA, University California, Davis. Characteristics: crisphead type, firm, good weight, smooth butt, large cores, attractive, low seedstalk, white seed. Resistance: susceptible to russet spotting, tipburn; contains PI 91532, downy mildew resistant Lactuca serriola introduction from Russia. 1978.

Velvet - Breeder: Vineland Agr. Expt. Sta., Ont., Canada. Vendor: Stokes Seeds, Ltd. Parentage: selected from unknown English cultivar. Characteristics: early dark green, very mild butterhead type. Resistance: bolting. Similar: Bibb. Rpt. Hort. Expt. Sta. 1955-56. p. 61. 1956.

Vista - Vendor: Vilmorin. Characteristics: cropping in spring, summer, and autumn, shiny medium green foliage, round heads, good shelf life, good clean underside without off-shoots. Resistance: Bremia R8, R11; tipburn, lettuce mosaic virus. Similar: Dolly. 1985.

Vista Verde - Breeder and vendor: Central Valley Seeds. Characteristics: crisphead type, very large frame and head, medium thick dark green leaves, medium pointed butt, narrow leaf petioles, high rib, black seed. Resistance: bolting, pink rib; tolerance to frost; not tolerance to heat. Similar: Vanguard. 1990.

Vitegra (29704) - Breeder and vendor: Enza Zaden. Parentage: open pollinated. Adaptation: North America. 1997.

Vulcan - Breeder and vendor: Sakata. Characteristics: leaf type, dark red color with light green contrast. Similar: Standard Red Leaf. 1989.

Waldmann's Green - Breeder: John Waldmann, Cincinnati, Ohio. Vendor: Joseph Harris Co., Rochester, N.Y. Parentage: selected from mutation or outcross in Grand Rapids. Characteristics: darker green than most leaf lettuce, fast growing, upright. Resistance: tipburn. Similar: Grand Rapids. Adaptation: greenhouse and outdoor culture. Harris Catalog. 1958

Wallaby (EX 9200027) - Breeder and vendor: Seminis Vegetable Seeds-Asgrow. Characteristics: coastal iceberg for warmer planting areas on the coast; medium frame, medium head Salinas type with medium green color; sowing dates for the Salinas Valley are May 1 to July 30. Resistance: corky root. Similar: Magnum. Adaptation: Salinas Valley, California. 2002.

Warrior - Breeder and vendor: Asgrow. Characteristics: crisphead type, medium size for mid-summer harvest in coastal California areas, medium dark green, good head coverage. Resistance: downy mildew; pathotypes 1, 2, 3. Similar: Salinas. 1991.

Weatherby (SSC 30529) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Resistance: bolting and corky root. Similar: Pam's Island Cos. Adaptation: All major lettuce growing regions. 2001.

Wes Pak - Breeder and vendor: Ferry-Morse Seed Co., Mountain View, Calif. Parentage: selection from Regular Great Lakes. Characteristics: large heads, good plant color, more finely frilled leaf margins. Similar: Great Lakes. Adaptation: general use. 1957.

Western Red Leaf - Breeder and vendor: Brinker. Characteristics: leaf type, dark red. Similar: Deep Red, Red Leaf, Prizehead. 1989.

Westland - Breeder: Brinker-Orsetti. Vendor: Brinker. Characteristics: crisphead type, large head, dark green during winter growing season. Resistance: lettuce mosaic virus. Similar: Vanguard. 1994.

Wildfire - Breeder and vendor: Johnny Selected Seeds. Parentage: open pollinated. Characteristics: Mix of several lettuce cultivars from several suppliers. 2000.

Winchester (SSC 30531) - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Resistance: bolting. Similar: Pam's Island Cos. Adaptation: all major lettuce growing regions. 2001.

Winter Select (PS 06510783) - Breeder and vendor: Seminis Vegetable Seeds. Parentage: open pollinated. Characteristics: large frame, medium green iceberg type that is sure heading; smooth ribs and very vigorous habit, good weight and very finished bottom; refined appearance makes it attractive for the cello market; suggested sowing dates are October 15-25. Similar: Cibola, Grizzly. Adaptation: NAFTA - desert (Imperial Valley of California and Yuma, Arizona). 2004.

Winter Supreme - Breeder: Bruce Church. Vendor: Petoseed. Characteristics: crisphead type, very large frame and head, thick dark green leaves, black seed. Resistance: bolting, pink rib; moderate to tipburn, rusty rib, downy mildew California pathovar 3; tolerance to frost; susceptible to big vein. Similar: Climax. 1978.

Winterhaven - Breeder: Harnish-Brinker/Marvins Seeds. Vendor: Petoseed. Characteristics: crisphead type, large frame and head, thick dark green leaves, smooth ribs, black seed. Resistance: pink rib, tipburn, downy mildew California pathovar 3; tolerance to heat, frost; susceptible to big vein. Similar: Vanguard. 1974.

Winterhaven Select - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: crisphead type; 110-120 day maturity; uniform maturity, medium sized head of medium green color; developed to produce full sized heads in mid-winter desert conditions; vigorous growth, leaves with good texture, smooth ribs. Resistance: downy mildew California pathovar 3; tolerance to frost. Similar: Vanguard, Winterhaven. Adaptation: California, Arizona. 1995.

Winterhaven Select - Breeder and vendor: Shamrock Seed Co. Parentage: open pollinated. Characteristics: crisphead type; 110-120 day maturity; uniform maturity, vigorous growth, large frame and head, dark dull green leaves, good texture, smooth ribs. Resistance: downy mildew California pathovar 3; tolerance to frost. Similar: Vanguard. 1995.

Winterhaven, BOS - Breeder: Brinker-Orsetti. Vendor: Orsetti. Characteristics: crisphead type, large frame and head, dull dark green leaves, medium large butt size, black seed. Resistance: bolting, tipburn; tolerance to heat, frost. Similar: Winterhaven. 1986.

Winterset - Breeder: E.J. Ryder. Vendor: USDA, ARS; Salinas, California. Characteristics: crisphead type, large frame and head, thick dull green leaves, large flat butt, slightly ribby, white seed. Resistance: tipburn, lettuce mosaic virus, limited downy mildew; tolerance to frost. Similar: Vanguard. 1984.

Wolverine (PS 5084 MI) - Breeder: Doug Sousa. Vendor: Seminis Vegetable Seeds-Petoseed. Parentage: open pollinated. Characteristics: late winter harvested crisphead lettuce, develops a thick leaf texture, good color, excellent cell pack appearance. Adaptation: desert southwest U.S. 1998.

Xena (HMX 6552) - Breeder: Clause. Vendor: Harris Moran Seed Co. Parentage: open pollinated. Characteristics: green leaf large head; glossy green leaf. Resistance: tipburn,

tolerance to bolting. Similar: Two Star. Adaptation: all loose leaf areas.

Yuma - Breeder: Harris Moran. Vendor: Harris Moran. Characteristics: crisphead type, large frame and head, dark green leaves, large butt. Resistance: fair to tipburn. 1985.

Yuri (RX 06511530) - Breeder and vendor: Seminis Vegetable Seeds-Royal Sluis. Parentage: inbred. Characteristics: Iceberg lettuce suitable for moderate to high temperature and humidity; Vanguard type, large frame, medium green color, round shape, with good bolting tolerance; recommended sowing period in Brazil is 1 March though 25 June; sowing period in the Caribbean is September through November; performed very well in elevations between 600 and 1500 m. Similar: Legacy. Adaptation: Brazil. 2003.

Zesty - Breeder: Ken Bixby. Vendor: Asgrow. Characteristics: cos type, dark green. Resistance: lettuce mosaic virus, downy mildew. Similar: Valmaine. 1987.

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The Use of Green Fluorescent Protein-Tagged Recombinant Viruses to Test *Lettuce mosaic virus* Resistance in Lettuce

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ABSTRACT

Candresse, T., Le Gall, O., Maisonneuve, B., German-Retana, S., and Redondo, E. 2002. The use of green fluorescent protein-tagged recombinant viruses to test *Lettuce mosaic virus* resistance in lettuce. Phytopathology 92:169-176.

Seed certification and the use of cultivars containing one of two, probably allelic, recessive genes, *mo1*^{1} and *mo1*^{2}, are the principal control methods for *Lettuce mosaic virus* (LMV) in lettuce. Although for a few LMV isolates, *mo1*^{2} confers resistance with most isolates, the genes *mo1*^{1} or *mo1*^{2} confer a tolerance, and virus accumulation is readily detected in *mo1*-carrying plants. This phenotype complicates evaluation of the resistance status, in particular for *mo1*^{1}, for which there are no viral strains against which a true resistance is expressed. Two green fluorescent protein (GFP)-tagged viruses were constructed, derived from a non-resistance breaking isolate (LMV-0) and from a resistance-breaking isolate (LMV-E). An evaluation of 101 cultivars of known status was carried out with these recombinant viruses. Using the LMV-0-derived recombinant, identification of *mo1*-carrying cultivars was simple because, contrary to its wild-type parent, systemic movement of LMV-0-GFP was abolished in resistant plants. This assay detected four cases of misidentification of resistance status. In all these cases, further tests confirmed that the prior resistance status information was incorrect, so that a 100% correlation was observed between LMV-0-GFP behavior and the *mo1* resistance status. Similarly, the LMV-E-derived recombinant allowed the identification of *mo1*^{2} lettuce lines because its systemic movement was restricted in *mo1*^{2} lines but not in susceptible or in *mo1*^{1} lines. The tagged viruses were able to systemically invade another host, pea, irrespective of its resistance status against another member of the genus *Potyvirus*, *Pea seed-borne mosaic virus*. The use of these recombinant viruses could therefore greatly facilitate LMV resistance evaluation and speed up lettuce breeding programs.

Lettuce mosaic virus (LMV), a member of the genus *Potyvirus* (26,27), is probably the most detrimental virus on lettuce crops worldwide (3). The virus is currently controlled by two distinct approaches: the use of virus-free seeds (5,8) supplemented in California with weed control measures and with an annual lettuce-free period of 2 weeks or, in Europe, by cultivation of resistant cultivars (3,29). A dominant gene, *Mo2*, providing immunity to LMV infection has been identified in a few lettuce cultivars (15). However, this gene is not useful in breeding programs because it is ineffective against the majority of LMV isolates (2,3,18). The only two resistance genes currently used to protect lettuce crops worldwide are therefore two recessive genes, *mo1*^{1} and *mo1*^{2}. The *mo1*^{1} gene, formerly named *g*, was initially identified in cv. Gallega de Invierno (1,28). It was mostly used by European breeders and has now been introduced into all types of lettuce, including butterhead, batavia, looseleaf, crisphead, and cos lettuce (15). The *mo1*^{2} gene identified in PI 251245, an accession of *Lactuca sativa* from Egypt (20,21) and originally named *mo*, has mostly been used by North American breeders who introduced it into crisphead and cos types of lettuce (15). Initially considered identical (20), these genes were later shown to have different specificities and to be either very closely linked or allelic genes and therefore were renamed *mo1*^{1} and *mo1*^{2} (3,15).

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In lettuce, LMV causes a variety of symptoms, which include vein clearing, mosaic, mottling or necrosis of the leaves, leaf deformation, dwarfing, and defective heading (3). Symptoms are quite variable and depend on the cultivar, the environmental conditions, and the developmental stage at which the plant became infected. Although symptoms on leaves are usually easy to detect, they may be much less conspicuous on some plant types such as varieties containing anthocyanin pigments or batavia-type cultivars with light green and irregular leaves (3). For a few LMV isolates such as LMV-1 or LMV-9, which overcome *mo1*^{1}, the *mo1*^{2} allele confers a true resistance with no detectable virus accumulation in the resistant cultivars (2,3,18). However, with most isolates, such as LMV-0, *mo1*^{1} and *mo1*^{2} confer only tolerance and virus accumulation is usually observed in varieties bearing these genes (2,3,10,12,18,29). These genes can thus be considered both as resistance and tolerance genes, depending on the viral isolate to which they are confronted. The common usage is, however, to use the term resistance to describe them and we have kept this wording throughout this work, despite the fact that the term tolerance would be more appropriate to describe the interaction under most circumstances. Although the viral concentrations reached in *mo1*-carrying varieties may be lower than that observed in susceptible ones (14,15), this effect is not observed in some virus–cultivar combinations (2,18,29). As a result, with the exception of the use of LMV-1 or LMV-9 in *mo1*^{2} cultivars, enzyme-linked immunosorbent assay (ELISA) is not an efficient way to determine the resistance status, and visual assessment of symptoms may be a more effective way of distinguishing resistant from susceptible plants (10). However, depending on the genetic background and the particular resistance gene present, the symptoms induced on resistant cultivars by common strains of LMV may vary from no symptoms at all to some, usually faint, level of chlorosis, mottling, or blotching (2,3,10,15,18,29).

Because the symptom protection afforded by the resistance genes is partially incomplete and affected by both varietal type and environmental conditions, evaluation of resistance on the basis of symptomatology is often difficult to perform (10), requiring either field experiments (29) or repeated observations under controlled conditions in growth chambers (2,18). As a consequence, and as is also the case for incomplete recessive resistance to other potyviruses (9), evaluation of resistance to LMV can yield confusing results. For example, misidentification of the resistance status of some plants or inappropriate interpretation of segregation data may explain why, using some of the same mapping populations, Montesclaros et al. (12) were not able to map the *mo1*^{2} gene and concluded that resistance was probably controlled by more than one gene, whereas Irwin et al. (10) mapped the resistance unambiguously to a single locus. Studies on the resistance of cucumber to potyviruses, which show some of the same characteristics, have also resulted in differences in interpretation of the resistance status of cucumber lines or of the dominance status of resistance genes (9). Discrimination between the two resistance alleles *mo1*^{1} and *mo1*^{2} in lettuce is also complex and can currently only be achieved by inoculation of *mo1*^{1}-breaking isolates such as LMV-1 or LMV-9 (15,18).

In this study, we report on the use of a green fluorescent protein (GFP) gene-tagged LMV recombinant isolate (LMV-0-GFP) to facilitate the screening of lettuce plants for LMV resistance and the use of a similarly tagged, resistance-breaking isolate (LMV-E-GFP) for the identification of the resistance alleles present in a particular variety.

MATERIALS AND METHODS

Plant materials. Seeds of lettuce cultivars were obtained from the collection of B. Maisonneuve or were provided either by commercial companies or by E. J. Ryder (USDA, Salinas, CA). All experiments were performed in a standard glasshouse (with additional light from 400 W sodium vapor pressure lamps) and in conformity with the requirements of the French Commission du Génie Génétique for the confinement of recombinant viruses. Lettuce seeds were germinated in sterilized soil and transplanted into 6-cm square pots. Plants were watered by subirrigation and received a fertilizer solution weekly.

Seeds of pea cultivars or of plant introduction lines (PI) were provided by E. Johansen (Danish Institute of Agricultural Sciences, Fredericksburg, Denmark). They were grown and inoculated essentially as described above for the lettuce plants.

Virus isolates and recombinant viruses. Viruses were propagated in plants of susceptible cv. Trocadéro. The common LMV-0 (non-resistance breaking) (3,18,19) and the *mo1*^{1}-breaking isolates LMV-1 and LMV-9 (3,18) were used to verify the resistance status of 13 cultivars.

The GFP-tagged recombinant LMV-E isolate (LMV-E-GFP) used in this study has previously been described (7). This recombinant was derived from LMV-E; an isolate able to overcome the resistance conferred by both the *mo1*^{1} and the *mo1*^{2} resistance genes (3,15,18,19). The GFP was inserted in frame in the viral polyprotein and is expressed as a translational fusion to the viral helper component (HC-Pro) protein.

A similar recombinant (LMV-0-GFP) was prepared from an infectious cDNA clone of LMV-0 (15,18,19,31) by inserting the GFP gene in the same manner in the viral genome. Both recombinant viruses were initially inoculated in cDNA form to plants of susceptible cv. Trocadéro by particle bombardment (31) and were later propagated in virus form by monthly passages in the same variety.

Virus propagation and resistance assays. Viral isolates were mechanically propagated as previously described (7,18) by rubbing two developed leaves on each plant with a suspension obtained by grinding 1 g of infected symptomatic lettuce leaves in 5 ml of inoculation buffer (50 mM Na_2HPO_4 and 0.2% sodium diethyldithiocarbamate) and adding 90 mg/ml of activated charcoal and 90 mg/ml of Carborundum. Following mechanical inoculation, plants were briefly rinsed with tap water. Usually four plants were inoculated per variety for the recombinant virus resistance assays and three plants for the natural viral isolates inoculations.

Plants were observed at frequent intervals for symptom development. GFP fluorescence was detected in a dark room or in the greenhouse at night with a 100 W hand-held long wave UV spotlight (Model B-100; UV Products, Upland, CA) (7).

RESULTS

Use of LMV-0-GFP in LMV resistance assays. In a preliminary experiment, the recombinant LMV-0 isolate expressing GFP as a fusion to HC-Pro (7) was inoculated to a differential set of lettuce cultivars (18). This initial experiment showed that LMV-0-GFP readily invaded susceptible cultivars such as 'Trocadéro' and could readily be detected in these plants by the high level of GFP fluorescence expressed (Fig. 1A and B). In contrast, no fluorescence was observed on leaves of three resistant cultivars, one harboring *mo1*^{1} ('Mantilia') and the other two *mo1*^{2} ('Vanguard 75' [22] and 'Salinas 88' [23]) (Fig. 1F). A large assay involving a number of susceptible and resistant (*mo1*^{1} or *mo1*^{2}) lettuce cultivars of different lettuce types, including varieties with green, yellow, and red leaves, was therefore performed to determine if inoculation with LMV-0-GFP could be used to differentiate susceptible and resistant lettuce cultivars.

In total, 101 cultivars of known resistance status were inoculated with LMV-0-GFP in seven successive independent experiments. Some cultivars were only tested once, whereas others were assayed in two or three different experiments. All inoculations and fluorescence notations were performed as blind tests under code numbers. The varieties tested included 48 butterhead cultivars (23 susceptible and 25 resistant), seven loose-leaf cultivars (five susceptible and two resistant), 29 batavia or crisphead cultivars (9 susceptible and 20 resistant), and 17 cos or Latin-type cultivars (4 susceptible and 13 resistant). The results obtained are presented in Table 1, and representative pictures illustrating the accumulation of GFP are presented in Figure 1.

Generally speaking, an excellent correlation was observed between the known resistance status of the cultivars and the detection of GFP fluorescence on the upper leaves of the inoculated plants. As reported previously for LMV-E-GFP (7), systemic invasion of the plants was rapid, with a timing comparable to that observed for wild-type virus. As a consequence, the values shown in Table 1 are derived from notations performed 6 to 9 days postinoculation (dpi), with the exception of experiment 5 for which notation was at 18 dpi. The notations (presence or absence of fluorescence on upper noninoculated leaves) were changed by observations performed at later times in only three cases out of a total of approximately 500 inoculated plants. These include observation of fluorescence on one susceptible 'Trocadéro' plant at 11 dpi but not at 8 dpi (experiment 4), and observation at 19 dpi (but not at 12 dpi, experiment 2) and at 11 dpi (but not at 8 dpi, experiment 4) of very weak and localized fluorescence on one or two upper leaves each of resistant cvs. Titan (Table 1) and Krizet (Table 1; Fig. 1E).

Fast systemic movement of LMV-0-GFP occurred in 39 of the 40 known susceptible cultivars studied, and GFP fluorescence was readily detected with a simple hand-held UV spotlight. Usually, all three or four tested plants showed the same behavior, but in two of the experiments (experiments 3 and 4), one (in two cases two) of the four inoculated plants did not show GFP accumulation for 10 cultivars from different types. Because in all cases in which these cultivars were tested twice a homogeneous behavior was observed in the other experiment, these plants most likely represent escapes from the inoculation procedure.

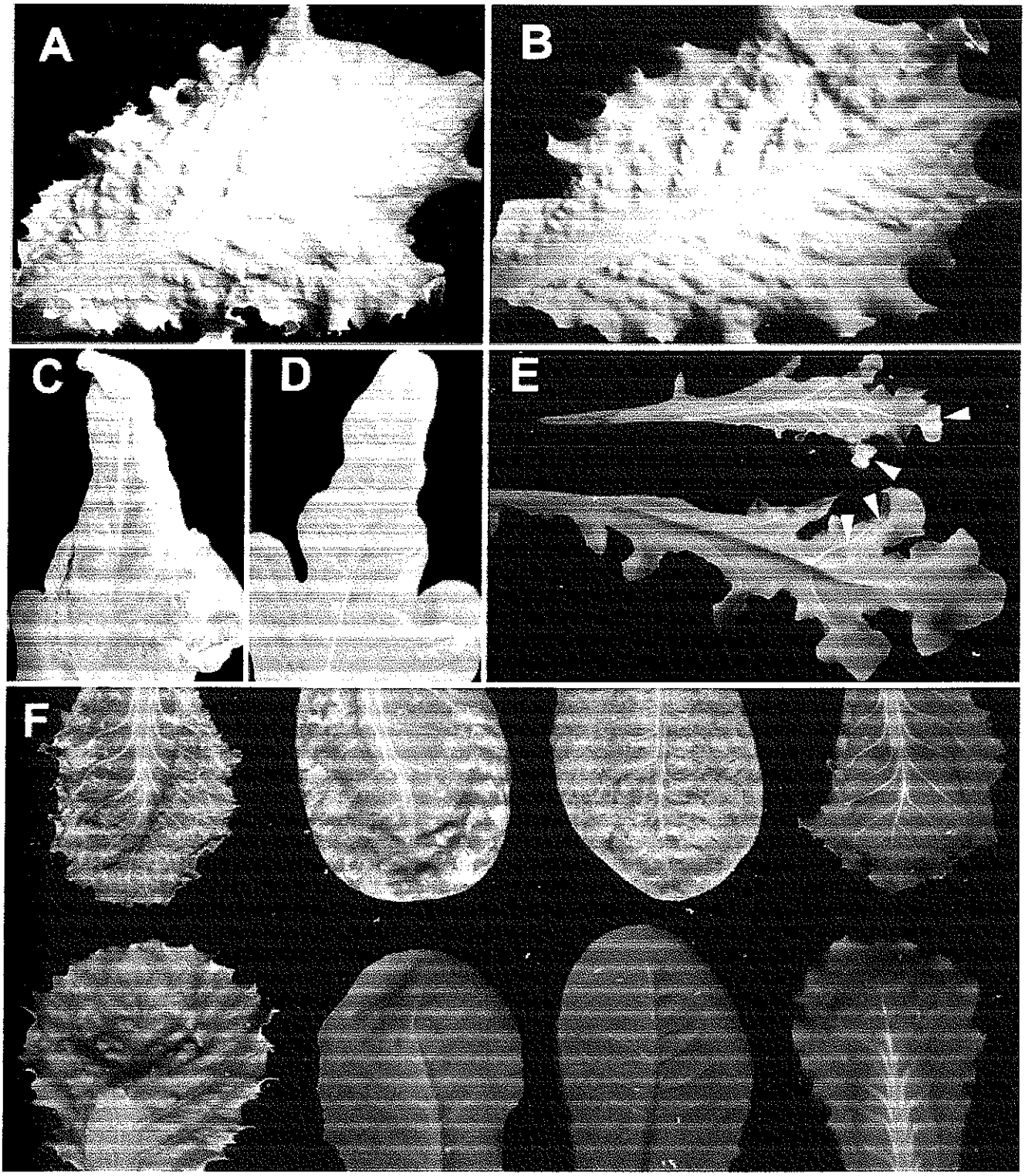

Fig. 1. Symptoms and systemic green fluorescent protein (GFP) accumulation upon inoculation of lettuce plants with recombinant *Lettuce mosaic virus* (LMV) isolates. **A,** Accumulation of GFP in a noninoculated leaf of butterhead cv. Angie upon inoculation of LMV-0-GFP. The GFP is green fluorescence on the red background resulting from chlorophyll autofluorescence. Picture taken 14 days postinoculation. **B,** Healthy control leaf of cv. Angie observed under identical conditions as for **A**. **C,** Necrotic symptoms induced in cv. Brunia by LMV-0-GFP. Picture taken 10 days postinoculation. **D,** Veinclearing symptoms induced in cv. Brunia by nonrecombinant LMV-0. Picture taken 10 days postinoculation. **E,** Weak GFP fluorescence detected in two leaves of a plant of cv. Krizet (arrows) 14 days after inoculation with LMV-0-GFP (Table 1). **F,** Detection of GFP accumulation in leaves of lettuce cultivars with different resistance status upon inoculation of LMV-E-GFP (top row) or of LMV-0-GFP (bottom row). The cultivars used from left to right: 'Trocadéro' (susceptible), 'Mantilia' (*mo1*^{1} resistant), 'Floribibb' (*mo1*^{1} resistant), and 'Vanguard 75' (*mo1*^{2} resistant). Picture taken 12 days after inoculation.

50

For the susceptible batavia lettuce, in which no systemic accumulation of GFP was observed (variety coded A, Table 1), further tests were performed with the non-resistance breaking isolate LMV-0 (18). The absence of symptoms indicated a wrongly determined resistance status and showed this cultivar to be in fact resistant to LMV (Table 1).

In the 60 known resistant cultivars, systemic accumulation of GFP was only observed in five cases. This included the late and very localized GFP detection in one leaf of one plant each of cvs. Titan (Table 1) and Krizet (Table 1; Fig. 1E) mentioned previously and the fast and effective accumulation of GFP observed in all tested plants of crisphead cv. Fame (Table 1) and cos cvs. Floricrisp and Marquis (Table 1). For these last three cultivars, full susceptibility to LMV infection was confirmed by inoculation with LMV-0 (Table 1).

A complete correlation between the LMV resistance status and the behavior of the LMV-0-GFP recombinant virus was observed (Fig. 1F). Consequently, inoculation with this GFP-tagged recombinant virus derived from LMV-0 provided a fast, simple and reliable way to evaluate the resistance of lettuce cultivars to LMV.

TABLE 1. Systemic green fluorescent protein (GFP) accumulation and *Lettuce mosaic virus* (LMV)-0 infection symptoms in butterhead lettuce cultivars and leaf, batavia and crisphead, and cos and Latin-type lettuce cultivars inoculated with LMV-0-GFP or LMV-0

Cultivar	Company[a]	R status[b]	LMV-0-GFP[c]							LMV-0[d]
			1	2	3	4	5	6	7	
Butterhead lettuce										
Alès	Vilmorin	S	...	4/4	...	...	...	...	3/3	3/3
Angie	Rijk Zwaan	S	...	...	3/4	...	...	...	3/3	3/3
Ariane	Rijk Zwaan	S	...	...	3/4	...	...	...	3/3	3/3
Columbus	Tézier	S	...	...	4/4	...	...	...	3/3	3/3
Fiona	Gautier	S	...	...	4/4	...	...	...	3/3	3/3
Flandria	Rijk Zwaan	S	...	4/4	...	...	...	...	3/3	3/3
Jessy	S & G	S	...	4/4	...	...	...	...	3/3	3/3
Johana	S & G	S	...	...	3/4	...	...	...	3/3	3/3
Judy	S & G	S	4/4	...	...	...	...	...	3/3	3/3
Magdalena	S & G	S	...	4/4	...	...	...	...	3/3	3/3
Manita	Rijk Zwaan	S	...	...	3/4	...	...	...	3/3	3/3
Mariska	Numhens	S	4/4	...	...	...	...	...	3/3	3/3
Méliès	Vilmorin	S	...	...	3/4	4/4	...	...	3/3	3/3
Ninja	S & G	S	...	...	3/4	...	...	...	3/3	3/3
Parade	Rijk Zwaan	S	...	4/4	...	...	...	...	...	...
Pégase	Vilmorin	S	...	4/4	...	...	...	...	3/3	3/3
Ramona	S & G	S	...	4/4	...	...	...	...	3/3	3/3
Salomé	Gautier	S	...	4/4	...	...	...	...	3/3	3/3
Samouraï	Rijk Zwaan	S	4/4	...	...	...	...	...	3/3	3/3
Sensai	Rijk Zwaan	S	...	...	2/4	...	...	...	3/3	3/3
Trocadéro	Old variety	S	...	...	...	3/4 (4/4[e])	...	...	3/3	3/3
Tzigane	Rijk Zwaan	S	...	4/4	...	...	...	...	3/3	3/3
Verpia	Clause	S	...	...	4/4	...	...	...	3/3	3/3
Alizé	Gautier	R	...	...	0/4	0/4	...	...	...	...
Alto	Rijk Zwaan	R	...	0/4	...	0/4	...	...	...	...
Audran	S & G	R	0/4	...	...	...	...	...	...	...
Augusta	Clause	R	...	0/4	0/4	...	...	...	...	0/3
Daguan	S & G	R	...	0/4	...	...	...	...	...	0/3
Divina	Vilmorin	R	...	0/4	...	...	...	...	...	...
Enrica	S & G	R	...	0/4	...	...	...	...	...	...
Ermosa	Gautier	R	...	0/4	...	...	...	...	...	0/3
Locness	Vilmorin	R	...	0/4	...	...	...	...	...	...
Lutine	Tézier	R	...	0/4	...	...	...	...	...	...
Magda	Vilmorin	R	...	0/4	...	...	...	...	...	...
Mantilia	S & G	R	0/4	...	...	0/4	...	...	...	...
Mirian	S & G	R	...	...	0/4	...	...	...	...	...
Nadine	Rijk Zwaan	R	0/4	...	...	...	...	...	...	...
Novir	INRA	R	...	0/4	...	...	...	...	...	...
Oriana	Gautier	R	...	...	0/4	...	...	...	...	...
Prestine	Tézier	R	...	0/4	0/4	...	...	...	...	0/3
Princess	Vilmorin	R	...	0/4	0/4	...	...	...	...	0/3
Remco	Rijk Zwaan	R	...	0/4	...	...	...	...	...	...
Salad Bibb	Harris Moran	R	...	...	...	...	0/3	...	...	...

(*continued on next page*)

[a] Vilmorin (La Ménitre, France), FAES (Florida Agricultural Experimental Station (Belle Glade), Rijk Zwaan (Aramon, France), Tézier (Portes les Valence, France), Gautier (Graines Gautier, Eyragues, France), S & G (Sluis & Groot, Les Ponts de Ce, France), Numhems (Semences Numhens, Sucelles, France), Clause (Brétigny sur Orge, France), INRA (Institut National de la Recherche Agronomique, Avignon, France), Harris Moran (Modesto, CA), Enza Zaden (Allones, France), USDA (U.S. Department of Agriculture, Salinas, CA), Asgrow (Garons, France), Arco (Newton Square, PA), Ferry Morse (Fulton, KY), and Petoseed (Garons, France).

[b] Reported resistance status: S = susceptible; R = resistant.

[c] Number of plants showing systemic GFP accumulation/number of LMV-0-GFP-inoculated plants. Six independent experiments were performed and are indicated by 1 to 5 and 7. Notations were performed 6 (experiment 2), 8 (experiments 4 and 6), 9 (experiments 1, 3, and 7), and 18 days (experiment 5) after inoculation.

[d] Number of plants with symptoms at 6, 8, or 9 days after inoculation/number of LMV-0-inoculated plants.

[e] One plant showing GFP accumulation 11 but not 8 days after inoculation.

[f] One plant showing low GFP accumulation on one leaf 19 days after inoculation.

[g] Very severe and necrotic symptoms.

[h] Plant showing very low GFP accumulation on one leaf 11 days after inoculation (Fig. 1E).

As previously reported for LMV-E-GFP (7), the symptoms induced by LMV-0-GFP were significantly milder than those induced by the parental LMV-0 isolate (data not shown). This was true for all varietal types and cultivars tested with a single notable exception. In looseleaf cv. Brunia, symptoms induced by LMV-0-GFP were considerably stronger than the mosaic induced by LMV-0 (Fig. 1C versus D). These symptoms included very severe stunting and leaf deformation as well as extensive necrosis (Fig. 1C). Necrotic lesions were also frequently observed on inoculated leaves. The same pattern was observed again when 'Brunia' was inoculated with LMV-E-GFP and LMV-E: symptoms induced by the GFP-tagged recombinant were more severe and strongly necrotic compared with the symptoms induced by the nonrecombinant parent (data not shown). This peculiar infection phenotype therefore appears to be linked to the tagging of the HC-Pro with GFP, a fact that was confirmed by the observation that neither LMV-0-GUS nor LMV-E-GUS (7) shows this increased severity phenotype in 'Brunia' (data not shown).

TABLE 1. *(continued from preceding page)*

Cultivar	Company[a]	R status[b]	LMV-0-GFP[c] 1	2	3	4	5	6	7	LMV-0[d]
Sangria	Vilmorin	R	...	...	0/4	...	...	...	...	...
Titan	S & G	R	...	0/4 (1/4[f])	...	...	...	...	...	0/3
Verian	S & G	R	...	0/4	...	...	...	...	...	...
F5 line in selection	INRA	R	...	0/4	...	...	...	...	...	...
F6 line in selection	INRA	R	...	0/4	...	...	...	...	...	0/3
Leaf lettuce										
Brunia	Vilmorin	S	...	8/8[g]	...	...	...	...	3/3	2/2
Cocarde	Gautier	S	4/4	...	...	...	...	...	1/1	1/1
Feuille de chêne blonde	Old variety	S	4/4	...	...	...	...	...	3/3	3/3
Magalie	Rijk Zwaan	S	...	4/4	...	...	...	...	3/3	3/3
Sigla	Enza Zaden	?	...	4/4	...	...	...	4/4	...	4/7
Kristine	Rijk Zwaan	R	0/4	...	...	...	...	...	...	...
Krizet	Rijk Zwaan	R	...	...	0/4	0/4 (1/4[h])	...	...	...	...
Batavia and crisp lettuce										
Coded A	Gautier	S	...	0/4	0/4	...	...	...	...	0/6
Dorée de printemps	Gautier	S	...	...	3/4	...	...	...	3/3	3/3
Floréal	Rijk Zwaan	S	...	...	3/4	...	...	...	3/3	3/3
Padana	Clause	S	...	4/4	...	...	...	...	...	2/2
Reines des glaces	Old variety	S	...	4/4	...	...	...	...	3/3	3/3
Salinas	USDA	S	4/4	...	...	2/4	...	...	3/3	3/3
Salvina	Clause	S	...	4/4	...	...	...	...	3/3	3/3
Vanguard	USDA	S	4/4	...	...	4/4	...	...	3/3	3/3
Verdie	Rijk Zwaan	S	...	4/4	...	...	...	...	3/3	3/3
Aida	S & G	R	...	...	0/4	0/4	...	...	...	...
Autumn Gold	USDA	R	...	...	...	...	0/3	...	...	...
Canasta	S & G	R	0/4	...	...	...	...	...	...	...
Carioca	Clause	R	...	...	0/4	...	...	...	...	...
Carmen	Gautier	R	...	0/4	...	...	...	...	...	0/3
Classic	Asgrow	R	...	...	...	...	...	0/4	...	0/4
Desert storm	Harris Moran	R	...	...	...	...	...	0/4	...	0/4
Dorémi	S & G	R	...	0/4	...	...	...	...	...	...
Fame	Asgrow	R	...	...	...	...	3/3	4/4	...	4/4
Kristia	S & G	R	...	0/4	...	...	...	...	...	...
Malika	S & G	R	0/4	...	...	...	...	...	...	...
Masaida	S & G	R	0/4	...	...	...	...	...	...	...
Mojave	Harris Moran	R	...	...	...		0/3	...	...	...
Nevada	Vilmorin	R	...	0/4	0/4	...	...	...	...	0/3
Panthéon	Rijk Zwaan	R	...	0/4	...	...	...	...	...	...
Rossia	Clause	R	...	0/4	0/4	...	...	...	...	0/3
Salinas 88	USDA	R	0/4	...	...	0/4	...	...	...	...
Sierra	Vilmorin	R	...	0/4	...	...	...	...	...	...
Vanguard 75	USDA	R	0/4	...	...	0/4	...	...	...	...
Winterset	USDA	R	...	...	...	...	...	0/4	...	0/4
Cos/Latin lettuce										
Little Leprechaun	Arco	S	...	4/4	...	...	...	...	3/3	3/3
Parris Island Cos	Ferry Morse	S	...	4/4	4/4	...	...	...	...	7/7
Remus	Rijk Zwaan	S	...	4/4	...	4/4	...	...	3/3	3/3
Ruby	USDA	S	...	4/4	...	...	...	...	3/3	3/3
Augustus	Petoseed	R	...	...	...	...	0/3	...	...	...
Floribibb	FAES	R	...	...	0/4	0/4	...	...	...	...
Floricos	FAES	R	...	...	0/4	0/4	...	...	...	...
Floricrisp	FAES	R	...	4/4	4/4	...	...	...	...	6/6
Floriglade	FAES	R	...	...	0/4	0/4	...	...	...	...
Gallega de Invierno	Old variety	R	...	...	0/8	...	...	...	...	0/3
Marquis	Asgrow	R	...	...	...	...	3/3	4/4	...	4/4
Plato	Petoseed	R	...	...	...	...	0/3	...	...	...
Presidio	Asgrow	R	...	...	...	...	0/3	...	...	...
Romulus	Petoseed	R	...	...	...	...	0/3	...	...	...
Short Guzmaine	FAES	R	...	0/4	...	0/4	...	...	...	...
F10 line in selection	INRA	R	...	...	0/4	...	...	...	...	...
F6 line in selection	INRA	R	...	...	0/4	0/4	...	...	...	...

Differentiation of *mo1*^{1} and *mo1*^{2} resistance alleles by LMV-E-GFP. LMV-E is a resistance-breaking isolate able to overcome resistance conferred by the *mo1*^{1} or the *mo1*^{2} genes (3,18). However, previous results (7) showed that GFP-tagging of this isolate resulted in an almost complete inability of the virus to move systemically in cv. Vanguard 75, which harbors the *mo1*^{2} resistance gene (22) (Fig. 1F). In fact, although some systemic movement and limited GFP accumulation was erratically and very occasionally observed (7), most 'Vanguard 75' plants inoculated with LMV-E-GFP failed to show GFP accumulation even 1 or 2 months after inoculation (31, this work). However, later tests showed that LMV-E-GFP was able to move readily in plants of cv. Mantilia, carrying the *mo1*^{1} gene (Fig. 1F). This observation indicated that LMV-E-GFP could not be used to discriminate between resistant and susceptible cultivars but suggested a series of experiments to determine whether LMV-E-GFP could be used to differentiate varieties carrying the *mo1*^{1} and *mo1*^{2} resistance genes. The results of these experiments are summarized in Table 2.

In total, 36 cultivars representing different varietal types were used in these experiments. These included three susceptible varieties, 23 *mo1*^{1}, and nine *mo1*^{2} varieties. The nature of the allele present in cv. Floribibb was unknown because its ancestry contained both *mo1*^{1} and *mo1*^{2} carrying lines (E. J. Ryder, *personal communication*). As for the experiments with LMV-0-GFP reported previously, systemic movement of LMV-E-GFP and GFP accumulation occurred rapidly and initial observations (performed 8 or 9 days after inoculation) were only very occasionally modified by observations at later times.

In all three susceptible cultivar (as well as in numerous others tested in different experiments, data not shown), systemic movement of LMV-E-GFP and GFP accumulation was readily observed (Fig. 1F). This was also the case in all *mo1*^{1}-carrying varieties, although 18 of 228 inoculated plants (nine cultivars) apparently escaped infection (Table 2). The fact that these varieties carry *mo1*^{1} and not *mo1*^{2} was confirmed for some of them by inoculation with *mo1*^{1}-breaking LMV isolates LMV-1 or LMV-9 (3,18). The resistance gene carried by cv. Floribibb was indicated as *mo1*^{1} by the ability of LMV-E-GFP to move systemically in this cultivar (Fig. 1F). This identification was confirmed by the development of symptoms upon inoculation of plants of this variety with LMV-1 (Table 2).

In contrast and similar to what had previously been observed in cv. Vanguard 75 (7), no systemic movement or GFP accumulation was observed in the nine cultivars carrying the *mo1*^{2} gene, even though some of the plants tested belonged to different varietal types (Table 2; Fig. 1F). Again, the presence of the *mo1*^{2} allele was confirmed for the varieties for which some doubts existed due to incomplete information (24) by their resistance to the *mo1*^{1}-breaking isolates LMV-1 or LMV-9 (Table 2).

The use of the LMV-E-GFP recombinant virus therefore provided a fast and straightforward way to differentiate lettuce

TABLE 2. Systemic green fluorescent protein (GFP) accumulation and *Lettuce mosaic virus* (LMV) symptoms on plants inoculated with LMV-0-GFP, LMV-E-GFP, or LMV-1/LMV-9

Cultivar	Resistance status[a]	Resistance gene	LMV-0-GFP[b]	LMV-E-GFP[c]	LMV-9 or LMV-1[d]
Salinas	S	None	9/11	4/4	nt
Trocadéro	S	None	7/7	4/4	4/4
Vanguard	S	None	11/11	4/4	nt
Aida	R	*mo1*^{1}	0/8	4/8	nt
Alizé	R	*mo1*^{1}	0/8	8/8	nt
Alto	R	*mo1*^{1}	0/8	2/4	nt
Augusta	R	*mo1*^{1}	0/8	2/4	nt
Augustus	R	*mo1*^{1}	0/3	3/3	2/2
Carioca	R	*mo1*^{1}	0/4	4/4	nt
Floribibb	R	?	0/8	6/8	4/4
Floricos	R	*mo1*^{1}	0/8	5/8	4/4
Floriglade	R	*mo1*^{1}	0/8	8/8	4/4
Gallega de Invierno	R	*mo1*^{1}	0/8	7/8	nt
Krizet	R	*mo1*^{1}	0/8 (1/8*)	8/8	nt
Mantilia	R	*mo1*^{1}	0/8	4/4	4/4
Mirian	R	*mo1*^{1}	0/4	3/4	nt
Nevada	R	*mo1*^{1}	0/8	4/4	nt
Oriana	R	*mo1*^{1}	0/4	2/4	nt
Plato	R	*mo1*^{1}	0/3	3/3	2/2
Presidio	R	*mo1*^{1}	0/3	3/3	2/2
Prestine	R	*mo1*^{1}	0/8	4/4	nt
Princess	R	*mo1*^{1}	0/8	4/4	nt
Romulus	R	*mo1*^{1}	0/3	3/3	2/2
Rossia	R	*mo1*^{1}	0/8	4/4	nt
Sangria	R	*mo1*^{1}	0/4	4/4	nt
Short Guzmaine	R	*mo1*^{1}	0/8	3/4	4/4
F10 line in selection	R	*mo1*^{1}	0/4	4/4	nt
Autumn Gold	R	*mo1*^{2}	0/3	0/3	0/2
Desert storm	R	*mo1*^{2}	0/4	0/4	0/4
Classic	R	*mo1*^{2}	0/4	0/4	0/4
Mojave	R	*mo1*^{2}	0/3	0/3	0/2
Salad Bibb	R	*mo1*^{2}	0/3	0/3	0/2
Salinas 88	R	*mo1*^{2}	0/8	0/4	nt
Vanguard 75	R	*mo1*^{2}	0/8	0/4	nt
Winterset	R	*mo1*^{2}	0/4	0/4	0/4
F6 line in selection	R	*mo1*^{2}	0/8	0/8	nt

[a] Known resistance status: S = susceptible; R = resistant.

[b] Number of plants showing systemic GFP accumulation/number of LMV-0-GFP-inoculated plants. The value given corresponds to the cumulative results of Table 1. * Indicates plant negative at 8 days after inoculation but showing weak fluorescence on one leaf at 11 days after inoculation.

[c] Number of plants showing systemic GFP accumulation/number of LMV-E-GFP-inoculated plants. Notation performed at 8 or 9 days after inoculation. Three or four plants were used per experiment, and a value of eight inoculated plants indicates two separate experiments.

[d] Number of plants showing LMV symptoms/number of LMV-1- or LMV-9-inoculated plants. Notation performed 6 or 8 days after inoculation, and results are not different 14 days after inoculation. nt = not tested.

53

varieties carrying the *mo1*^{2} resistance gene from the susceptible varieties or from those carrying the *mo1*^{1} gene.

Systemic movement of LMV-0-GFP is not affected in pea. In order to evaluate whether the restriction of the movement LMV-0-GFP was specific to lettuce or could be also observed in another host, the behavior of the wild type and of the tagged virus were evaluated in pea. Pea has been described as a host of LMV but a screening of pea germ plasm failed to reveal significant resistance to LMV (16). However, numerous resistance genes, including recessive ones, have been described in pea against another member of the genus *Potyvirus*, *Pea seed-borne mosaic virus* (PSbMV) (13). Eleven varieties or PI lines carrying either no resistance to PSbMV or various combinations of PSbMV resistance genes (13) were therefore inoculated with LMV-0 and LMV-0-GFP and symptoms and/or GFP accumulation monitored. The results are presented in Table 3. As can be seen, systemic movement of both the wild type and the GFP-tagged viruses occurred in all pea plants tested, irrespective of their resistance status against PSbMV.

DISCUSSION

The two closely linked or allelic genes, *mo1*^{1} (15,28) and *mo1*^{2} (15,20), are currently used worldwide to protect lettuce crops against the detrimental effects of LMV infection. As is frequently observed for resistance to potyviruses (6,9), these genes are recessive and do not provide immunity to viral infection by common LMV strains. These genes restrict the long-distance movement of the virus (7) or affect symptom expression and/or viral accumulation (2,3,15,18,29). As a consequence of these incomplete resistance characteristics, evaluation of the resistance status cannot be performed reliably with ELISA assays (10) and thus relies on symptom observation, which may also be difficult because symptoms are affected both by environmental conditions and by lettuce varietal type (2,3,10,29). However, evaluation of *mo1*^{2} resistance can be simplified by the use of isolates such as LMV-1 or LMV-9 against which a complete resistance phenotype is observed. Given these difficulties or limitations, the development of a simpler and more reliable assay is highly desirable. The results presented here show that the use of the recombinant virus LMV-0-GFP expressing the GFP as a fusion with the HC-Pro provides just such a simple assay, particularly for the *mo1*^{1} gene.

The ability to tag potyviral genomes by insertion of a reporter gene between the P1 and HC-Pro proteins and expression of this reporter as a translational fusion to the HC-Pro was initially demonstrated with *Tobacco etch virus* (TEV) (4) and later extended to several other members of the genus (17). The use of such recombinant viruses expressing GUS, *bar* (glufosinate resistance), or a chloroplast-targeted cytochrome P450 for the screening of ecotypes or mutants of *Arabidopsis thaliana* (11,30) has already been reported in the case of TEV. Similarly, recombinant TEV isolates expressing the GUS gene were used to study the mechanism of the resistance to TEV of the V20 tobacco cultivar (25).

The recombinant LMV isolates used here were developed in a similar manner (7,31). These viruses showed attenuated symptoms in all lettuce hosts tested compared with the parental virus from which they were derived (7; this study), with the notable exception of their increased severity in cv. Brunia (Fig. 1C and D) for which there is currently no explanation. Although the GFP-tagged recombinants are quite stable (stability and GFP fluorescence have been observed throughout the entire life cycle of early flowering lines of lettuce), the GFP insert may sometimes be lost upon prolonged multiplication (7). The deleted viruses recovered no longer express GFP and may revert to more severe symptoms (7). Although the heterogeneous behavior of some lines observed upon recombinant virus infection during this study is most probably explained by inoculation escapes, demonstrated by the fact that homogeneous behavior was observed upon retesting, another possibility is that the susceptible plants failing to show fluorescence contained a recombinant that had lost the GFP insert. The third possibility, that the cultivars used were segregating for resistance is highly unlikely because the seeds used were from fixed commercial cultivars.

Because individual experiments reported here spanned about a 1-year period and were performed under standard glasshouse conditions with no specific efforts to reduce season-to-season variations in environmental conditions, the behavior of the recombinant viruses in the various lettuce types does not appear strongly influenced by environmental conditions. Similar to what happens with the wild-type virus, the time needed for systemic movement of the recombinant viruses and, therefore, for the visualization of the GFP fluorescence, may however be somewhat longer during the winter months. Tests can nevertheless be performed in glasshouses without the need for controlled-environment growth chambers. The only constraint is the need for a dark room or the obligation to perform evaluations at night so that daylight does not interfere with the observation of fluorescence under UV light illumination. In these conditions, observation of GFP fluorescence is very straightforward and can be performed on any varietal type or cultivar, even by untrained personnel. In addition, the recombinant viruses used in this study are easy to confine because GFP-tagging of the HC-Pro abolishes aphid transmissibility (7) as well as seed transmissibility (7; our own unpublished results with LMV-0-GFP).

There is currently no explanation for the restricted-movement behavior of LMV-0-GFP in *mo1* varieties or of LMV-E-GFP in *mo1*^{2}-carrying varieties. Both observations are surprising because in each case, the wild-type parental isolate is able to replicate and

TABLE 3. Systemic green fluorescent protein (GFP) accumulation and *Lettuce mosaic virus* (LMV) symptoms on pea plants inoculated with LMV-0-GFP or LMV-0

Cultivar	Resistance status[a]	Resistance gene[b]	LMV-0-GFP[c]	LMV-0[d]
Scout	S	None	3/3	3/3
Brutus	S	None	3/3	3/3
Fjord	S	None	3/3	3/3
Bonneville	R	*sbm2*	3/3	3/3
PI 347422	R	*sbm2/3*	3/3	3/3
DSP	R	*sbm2/3*	3/3	3/3
PI 347484	R	*sbm2/3*	3/3	3/3
PI 269774	R	*sbm1* + *sbm2/3*	3/3	3/3
PI 269818	R	*sbm1* + *sbm2/3*	3/3	3/3
PI 193586	R	*sbm1* + *sbm4* + *sbm2/3*	3/3	3/3
PI 347329	R	Seggregating for *sbm1* + *sbm4* + *sbm2/3*	3/3	3/3

[a] Known *Pea seed-borne mosaic virus* (PSbMV) resistance status: S = susceptible; R = resistant.
[b] PSbMV resistance genes present according to Olsen and Johansen (13).
[c] Number of plants showing systemic GFP accumulation/number of LMV-0-GFP-inoculated plants. Notation performed 11 days after inoculation.
[d] Number of plants showing LMV symptoms/number of LMV-0-inoculated plants. Notation performed 11 days after inoculation.

54

move systemically in susceptible as well as in resistant plants (3,18). This effect thus appears to be directly linked with the addition of an N-terminal translational fusion to the viral HC-Pro protein. Results with a similarly tagged LMV-E-GUS have shown that the failure to observe systemic viral movement in *mo1*^{2}-resistant cultivars such as 'Vanguard 75' (22) is due to a block in long-distance viral spread (7). Similarly, the very infrequent (two plants out of a total of more than 290 inoculated plants) and late observation of a few spots of LMV-0-GFP fluorescence on two resistant varieties demonstrate the ability of the recombinant to multiply and spread cell to cell in these plants and indicate that the block observed is very likely at the level of long-distance movement. In those two cases, the low amount of GFP observed was clearly different from the high and widely spread fluorescence observed in susceptible varieties (Fig. 1E), making confusion highly improbable when interpreting results.

The tagged viruses were inoculated to pea cultivars containing a range of recessive resistance genes against another member of the genus *Potyvirus*, PSbMV. In all plants and irrespective of their PSbMV-resistance status, the tagged viruses were able to move systemically, with a timing very similar to that of the wild-type parents. These results show that the modified accumulation pattern of the tagged viruses observed in *mo1*-carrying plants reflects a specific interaction with the *mo1* genes and is unlikely to represent a general property of the tagged viruses. Further evidence that the restricted movement phenotype of tagged LMV isolates in resistant lettuce cultivars may not apply to other *Potyvirus*–host combinations comes from results of a study showing that a resistance-breaking GUS-tagged TEV was able to move systemically in V20 resistant tobacco (25).

By allowing a more straightforward evaluation of resistance than symptom observation or lengthier ELISA assays, GFP-tagged viruses offer a new and powerful tool that should be useful in breeding and mapping projects. It is tempting to speculate on the generality of the observations reported here and on the possibility of developing recombinant viruses for the screening of genes that are difficult to assess because they afford incomplete resistance to potyviruses (9). Although only experimental work will allow the assessment of such a possibility, the results reported and results with GUS- or *bar*-tagged TEV (25,30) indicate that this is a distinct and promising possibility.

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Molecular characterization of *mo1*, a recessive gene associated with *Lettuce mosaic potyvirus* resistance in lettuce

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Abstract: Lettuce mosaic, caused by the potyvirus LMV, is a serious viral disease of lettuce crops world-wide. Beside seed control, one of the leading strategies to control LMV is the use of the recessive resistance genes *mo1*[1] and *mo1*[2]. In an attempt to characterize the functional role of the translation initiation factor eIF4E in lettuce susceptibility to LMV, we cloned *Ls-eIF4E* cDNAs from a collection of lettuce cultivars. Data including sequence co-variation among nineteen lettuce genotypes, genetic co-segregation and functional complementation using two different bio-assays indicated that *Ls-eIF4E* is *mo1*.

Keywords: *Lactuca sativa*, cap-binding protein, eIF4E, recessive resistance, host resistance, virus movement

Introduction

Genetic resistance is a major approach to control plant diseases in the field. Cloning plant genes associated with resistance to viruses or other pathogens gives insight into the mechanisms underlying the resistance phenotype, and will also inform the design of strategies to provide more durable resistance strategies. Several genes for Leucine-rich repeat (LRR) proteins belonging to one of several classes and controlling hypersensitivity-based resistance to various types of plant pathogens in a dominant manner, have been cloned in recent years (Pontier et al., 1998).

In the case of viruses and especially potyviruses, a number of resistance phenotypes used by breeders have been associated with recessive alleles of single genes (Provvidenti and Hampton, 1992). Resistance is therefore probably associated with other mechanism(s) than hypersensitivity. A working hypothesis is that recessive resistance genes encode host factors recruited by the virus, and that their debilitation by mutation or inactivation reduces the virus ability to complete its cycle. In several plant-potyvirus models, sequence variations in the viral protein called VPg have been associated with the success of certain virus isolates to infect and produce symptoms in plants normally protected by a recessive gene (Keller et al., 1998, Nicolas et al., 1997, Schaad et al., 1997). VP has been shown to interact *in vitro* with isoforms of the cap-binding eukaryotic translation initiation factor eIF4E (Léonard et al., 2000, Schaad et al., 2000, Wittmann et al., 1997).

We have investigated the role of eIF4E in the compatibility between a potyvirus, *Lettuce mosaic virus* (LMV) and its host plant, lettuce (*Lactuca sativa*), in relation with the presence of the apparently allelic recessive genes *mo1*[1] and *mo1*[2] (Dinant and Lot, 1992, Ryder, 1970).

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56

Material and methods

Plant material and viral constructs

The LMV susceptible lettuce genotypes Fiona, Girelle, Jessy, Mariska, Salinas, Vanguard, Trocadéro and 87-20M, the *mo1*^{1} genotypes Alizé, Classic, Floribibb, Malika, Mantilia, Oriana and Presidio and the *mo1*^{2} genotypes Autumn Gold, Desert Storm, Vanguard 75 and Salinas 88 (Candresse et al., 2002) were used. F1 hybrids between Mantilia on the one hand and Mariska or Girelle on the other hand were produced and self-pollinated to obtain F2 progenies. Plants were routinely grown under greenhouse conditions.

LMV-0-4E^0 and LMV-0-4E^1 were constructed to express the Ls-eIF4E^0 or Ls-eIF4E^1 cDNAs as *in vivo* processed translational fusions with the polyprotein of LMV-0, a common isolate of LMV. Symptoms were recorded 10 to 15 days after inoculation. Detection of the viral progeny was made by RT-PCR or DAS-ELISA. DAS-ELISA was performed after ten-times dilution of the plant extracts, so that the relationship between OD_{405} and antigen concentration was linear in our concentration range.

RT-PCR amplification, cloning and sequencing of cDNAs

Total cDNA was synthesized from total lettuce RNA using AMV Reverse Transcriptase, and PCR-amplified using *Taq* DNA Polymerase and synthetic oligonucleotide primers. The cDNA 5' and 3' ends were amplified using commercial RACE PCR kits. All amplified cDNAs were cloned in pGEM®-T Easy and sequenced using automated DNA sequencing.

Sequence analysis

The eIF4E and eIF(iso)4E sequences from a variety of plant and animal species were retrieved from GenBank. Multiple sequence alignments were generated using ClustalW (Thompson et al., 1994).

The 3D structures of cap-bound human and murine eIF4E were retrieved from PDB. Comparative protein modeling was elaborated online using Swiss-Model and Swiss-PdbViewer (Guex and Peitsch, 1997) and 3D-Jigsaw (Bates et al., 2001). The fit between 3D structure models was evaluated in Swiss-PdbViewer by calculating the root mean square (RMS) deviation (Chothia and Lesk, 1986) after iterative fitting.

Results and discussion

Cloning and sequence analysis of the lettuce eIF4E and eIF(iso)4E cDNAs

The central region of the *eIF4E* cDNA from the susceptible lettuce genotype Salinas was PCR-amplified using degenerate oligonucleotides derived from seven plant *eIF4E* sequences, including three from *A. thaliana*. The 169-bp sequence obtained was used to design new oligonucleotides for 3'RACE and 5'RACE amplification of the cDNA ends. The terminal sequences determined from the corresponding cDNAs served to design a pair of oligonucleotides allowing PCR-amplification of the nearly full-length *eIF4E* cDNA, including the entire coding region. The assembled full-length sequence encoded a 26 kD protein. The identity between the predicted amino-acid sequence and eleven eIF4E sequences from plants, vertebrates and insects ranged between 40 and 70% (data not shown). Similarly, a nearly full-length Salinas *eIF(iso)4E* cDNA encoded a 22 kD protein homologous to other plant *eIF(iso)4E*.

Correlation between mutations in the eIF4E cDNA and the presence of mo1^1 *or mo1*^{2}

The coding region of the *eIF4E* cDNA from seven additional lettuce genotypes (Floribibb, Mantilia, Malika, Salinas 88, Vanguard, Vanguard 75 and 87-20M) was PCR-amplified,

cloned and sequenced as described above. On the basis of the presence of few variations, these sequences could be classified into three types, Ls-eIF4E°, Ls-eIF4E^1 and Ls-eIF4E^2 (Table 1). There was a strict correlation between *Ls-eIF4E*^{1} and the presence of *mo1*^{1}, and between *Ls-eIF4E*^{2} and the presence of *mo1*^{2}, while the susceptible genotypes all had *Ls-eIF4E*°. This correlation was maintained even in the two independent pairs of genotypes nearly isogenic for *mo1*^{2}, Salinas / Salinas 88 and Vanguard / Vanguard 75. It was also confirmed and extended when the central domain of the *Ls-eIF4E* cDNA from eleven additional genotypes was sequenced: Fiona, Girelle, Jessy and Mariska (*Ls-eIF4E*°, susceptible), Alizé, Classic, Oriana, and Presidio (*Ls-eIF4E*^{1}, *mo1*^{1}), and Autumn Gold and Desert Storm (*Ls-eIF4E*^{2}, *mo1*^{2}) (not shown).

A three dimensional (3D) model of the Ls-eIF4E protein was predicted based on the known 3D structures of human and murine cap-bound eIF4E molecules (Marcotrigiano et al., 1999, Tomoo et al., 2002). The amino-acids that differ between the thre e Ls-eIF4E types were predicted to be at or near the surface of the protein, in two different loops near the cap-recognition pocket (not shown).

In contrast, no sequence difference was detected between the *eIF(iso)4E* sequences from Salinas and Vanguard and from their related *mo1*^{2} genotypes Salinas 88 and Vanguard 75. An identical sequence was also found in Mantilia (*mo1*^{1}). This suggests that eIF(iso)4E sequence variations are not directly linked with the *mo1*-related phenotypes.

Table 1. Three types of eIF4E sequences in lettuce. The nucleotide sequence differences found in seven genotypes compared to Salinas are shown, with their corresponding amino-acid differences. Position 730 is in the 3' non-coding region and therefore translation does not apply (n.a.).

		Nucleotide (Amino-Acid) positions									
		228 (70)		299 (93)		344-349 (108-110)		576 (186)		730 (n.a.)	
		nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
eIF4E°	Salinas Vanguard 87-20M	G	Ala	C	Phe	AGG- -AGC	QGA	G	Ala	C T	n.a.
eIF4E^1	Floribibb Malika Mantilia			T	Phe	(del)	His	T	Ser		
eIF4E^2	Salinas 88 Vanguard 75	C	Pro							T	n.a.

Genetic co-segregation between mo1^1 and Ls-eIF4E^1

The 6-nucleotide deletion found at positions 344-349 in *Ls-eIF4E*^{1} is associated with the presence of a *Pag*I restriction site, a variation that could easily be scored after PCR amplification of the central region of *Ls-eIF4E* and *Pag*I digestion. The segregations of this CAPS marker and of LMV resistance were analysed in parallel in two [susceptible x *mo1*^{1}] F2 progenies, [Mariska x Mantilia] and [Girelle x Mantilia]. Three distinct CAPS pattern were observed (Figure 1). All resistant plants were homozygous for the presence of *Pag*I. The susceptible plants were either homozygous for the absence of *Pag*I for about one third of them, or showed a partial digestion, and were thus identified as heterozygous for *Pag*I for the remaining two-thirds. Thus, a complete map co-segregation was observed between *mo1*^{1}-associated LMV resistance and the CAPS marker characteristic of *Ls-eIF4E*^{1}.

58

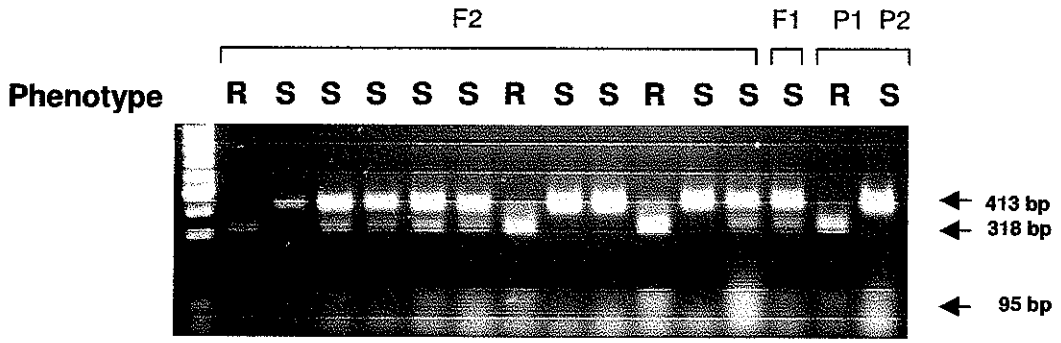

Figure 1. Co-segregation of *mo1*[1] and *Ls-eIF4E*[1]. RT-PCR products from *eIF4E* from Mantilia (P1), Mariska (P2), their F1 progeny and a set of F2 progenies were generated and digested with *Pag*I. The original 448-bp product is digested into a 413-bp product due to the presence of a conserved *Pag*I site in all genotypes, used as an internal control. The 413-bp product was further processed in *Ls-eIF4E*[1].

Ls-eIF4E°, but not Ls-eIF4E[1], restores the infectivity of a recombinant LMV in* mo1 *plants. LMV-0-4E° and LMV-0-4E[1] were inoculated to Trocadéro, Salinas and Vanguard (susceptible), Floribibb, Malika and Mantilia (*mo1*[1]), and Salinas 88 and Vanguard 75 (*mo1*[2]). As expected, LMV-0 caused symptoms only in the susceptible varieties (not shown). This was also the case of LMV-0-4E[1]. However, typical LMV symptoms appeared in all plants inoculated with LMV-0-4E°.

No accumulation of non-recombinant LMV-0 was detected in the *mo1*[1] plants tested by ELISA, and accumulation was strongly reduced in *mo1*[2] plants compared to susceptible plants (Table 2). The same situation was observed for LMV-0-4E[1]. On the other hand, LMV-0-4E° accumulated to similar levels in all three categories of genotypes.

Therefore, ectopic Ls-eIF4E expression can not only complement LMV accumulation in a normally resistant *mo1*[1] context, but also LMV symptom expression in a normally tolerant *mo1*[2] context, suggesting that resistance and tolerance, in this case, are functionnally related.

Agro-infiltration of Ls-eIF4E° in the upper, non-inoculated, leaves of Salinas 88 (*mo1*[2]) previously inoculated in their lower leaves with LMV-0-GFP restored the systemic accumulation of the virus in the infiltrated area (not shown). This was also observed for Ls-eIF4E[1] or Ls-eIF4E[2], but to a lesser extent (not shown), suggesting that quantitative expression could at least partly restore a qualitative defect of eIF4E.

Table 2. Virus accumulation was determined by ELISA 3 weeks after inoculation with LMV-0 containing the Ls-eIF4E° or Ls-eIF4E[1] coding sequence in two independent experiments. For each construct, the values were normalized to those obtained in the susceptible cultivar Salinas. LMV-0 and LMV-0-4E[1] were consistently not detectable in ELISA in Mantilia.

	Experiment 1		Experiment 2	
	LMV-0	LMV-0-4E°	LMV-0-4E°	LMV-0-4E[1]
Mantilia (*mo1*[1])	n.a.	97.1 ± 11.5	111.7 ± 20.5	n.a.
Salinas 88 (*mo1*[2])	8.7 ± 8.1	71.7 ± 26.0	105.7 ± 42.4	5.9 ± 11.0

Conclusions

Taken together, our results show not only a sequence co-variation and genetic co-segregation between *mo1* alleles and *eIF4E* in lettuce, but also functional complementation for LMV accumulation and symptom expression by ectopic expression. This strongly suggests that *Ls-eIF4E* is *mo1*. This is reminiscent of results recently obtained in pepper showing that the recessive PVY resistance gene *pvr2* is also *eIF4E* (Ruffel et al., in press), and that *eIF(iso)4E* disruption in *Arabidopsis thaliana* confers resistance to a variety of potyviruses including LMV (Duprat et al., in press, Lellis et al., 2002). This makes *eIF4E* the first natural recessive plant virus resistance gene cloned, and suggests that the large proportion of recessivity in resistance genes against potyviruses in crop plants (Provvidenti and Hampton, 1992) might reflect a conserved host-virus interaction mechanism still to be understood.

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60

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SNP-based codominant markers for a recessive gene conferring resistance to corky root rot (*Rhizomonas suberifaciens*) in lettuce (*Lactuca sativa*)

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Abstract: The analysis of F_2 progeny and derived F_3 families of *Lactuca sativa* segregating for resistance to corky root rot caused by *Rhizomonas suberifaciens* permitted the identification of restriction fragment length polymorphism (RFLP) and single nucleotide polymorphism (SNP) markers linked to the recessive resistance gene *cor*. PCR-based markers were identified by bulked segregant analysis (BSA). Allele-specific primers were generally designed with the 3′ terminal base coinciding with an SNP, matching one of the alleles and mismatching the other, and with an additional subterminal 3′ base mismatching both alleles. Codominant, robust, and inexpensive molecular markers were obtained that used standardized PCR conditions. Some of the markers could be analyzed in multiple *Lactuca* mapping populations that did not segregate for disease resistance allowing the *cor* locus to be located on several maps. The consistent low density of markers around *cor* in these maps suggests that *cor* may be in an area with an elevated rate of recombination. Evaluation of these markers in a large sample of cultivars and landraces identified pairs of flanking polymorphic markers that can be used for marker-assisted selection of corky root resistance.

Key words: single nucleotide polymorphism (SNP), sequence characterized amplified region (SCAR), marker-assisted selection (MAS), genetic map, resistance gene.

Résumé : L'analyse d'une progéniture F_2, ainsi que les familles F_3 qui en sont dérivées, du *Lactuca sativa* en ségrégation pour la résistance à la racine liégeuse, une maladie causée par le *Rhizomonas suberifaciens*, a permis d'identifier des marqueurs RFLP (« restriction fragment length polymorphism ») et SNP (« single nucleotide polymorphism ») liés au gène de résistance récessif *cor*. Des marqueurs de type PCR ont été identifiés par analyse des ségrégants en mélanges (BSA ; « bulked segregant analysis »). Des amorces allèle-spécifiques ont été conçues pour que la base terminale en 3′ corresponde au site SNP, c'est-à-dire que la base terminale soit parfaitement appariée chez l'un des allèles, mésappariée chez l'autre allèle et que l'avant dernière base soit mésappariée aux deux allèles. Des marqueurs codominants, robustes et peu coûteux ont été obtenus en faisant appel à des conditions d'amplification standardisées. Certains de ces marqueurs ont pu servir au sein de diverses populations de cartographie qui n'étaient pas en ségrégation pour la résistance à la maladie, ce qui a permis de cartographier le locus *cor* sur plusieurs cartes génétiques. La faible densité de marqueurs observée de manière répétée à proximité du locus *cor* sur ces cartes suggère que ce locus se trouverait dans une région qui présente un taux élevé de recombinaison. L'évaluation de ces marqueurs au sein d'un grand échantillon de cultivars et de variétés locales a permis d'identifier des paires de marqueurs polymorphes voisins qui peuvent servir en sélection assistée de marqueurs pour la résistance à la racine liégeuse.

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62

Mots clés : polymorphisme mononucléotidique (SNP), régions amplifiées de séquence connue (SCAR), sélection assistée de marqueurs (MAS), carte génétique, gène de résistance.

[Traduit par la Rédaction]

Introduction

Corky root rot (CR) is the second most important disease of lettuce (*Lactuca sativa* L.) in California and it has also been reported in Australia, Canada, and many countries in Europe (van Bruggen et al. 1989; Datnoff and Nagata 1992; van Bruggen and Jochimsen 1992). The causal agent is the Gram-negative bacterium *Rhizomonas suberifaciens* (van Bruggen et al. 1988). Susceptible cultivars develop yellow–green lesions on the taproot and main lateral roots, which may cover the entire root surface and become corky in texture. This impairs root function; plants may wilt, become stunted, and produce small unmarketable heads (van Bruggen et al. 1988). Partial disease control may be obtained with cultural practices (Patterson et al. 1981). Although fumigation with methyl bromide is effective (O'Brien and van Bruggen 1990), it is uneconomic and is being eliminated from use in many countries after the *Montreal Protocol on Substances that Deplete the Ozone Layer* (Sarma and Bankobeza 2000). The use of resistant cultivars is the optimal method for long-term control (Sequeira 1970; Brown and Michelmore 1988).

Resistance to *R. suberifaciens* was first identified in a landrace leaf lettuce accession from Turkey (PI171669) and introduced into commercial crisphead cultivars for the east coast of the U.S.A. using the crisphead lettuce *L. sativa* 'Oswego' as the recurrent parent. Three highly resistant crisphead lettuces, *L. sativa* 'Marquette', *L. sativa* 'Montello', and *L. sativa* 'Green Lake', were released (Dickson 1963; Sequeira 1970; Sequeira 1978). Since then, breeding efforts have been based almost entirely on the resistance derived from PI171669 (Brown and Michelmore 1988; Ryder and Waycott 1994). A germplasm screen of over 500 accessions of *L. sativa* and other *Lactuca* species for resistance to *R. suberifaciens* strain CA1 that causes CR in California identified resistance in several accessions of *Lactuca sativa*, *Lactuca serriola*, and *Lactuca saligna*. Genetic analysis demonstrated that resistance to strain CA1 was conferred by a single recessive gene; this gene was designated *cor* (Brown and Michelmore 1988). Genetic analysis failed to reveal additional loci for resistance (Brown and Michelmore 1988).

The severity of CR attack is sensitive to environmental changes, making phenotypic evaluation of resistance difficult and unreliable, even under controlled conditions in a growth chamber (Brown and Michelmore 1988). This, combined with the recessive nature of resistance, makes CR resistance an excellent candidate for breeding using marker-assisted selection. In an initial attempt to identify molecular markers linked to *cor*, an F_2 population was generated by crossing the highly susceptible crisphead lettuce 'Salinas' with PI491251, a highly resistant *L. serriola* accession identified in the germplasm screen. Analysis of this population identified three markers loosely linked to *cor*: *CL658* (the closest at 35 cM), *CL425*, and *OPHO3*. These markers were positioned on an intra-specific map for *L. sativa* 'Calmar' × *L. sativa* 'Kordaat' (Kesseli et al. 1994); however, *cor* could not be precisely located on the intra-specific map because no tightly linked markers were identified and both parents of this mapping population were susceptible to corky root (Kesseli et al. 1994; R. Kesseli and R. Michelmore, unpublished).

The first objective of the current study was to locate the resistance gene *cor* precisely on the genetic map of lettuce, circumventing the fact that none of the main mapping populations segregated for disease resistance. Secondly, we aimed to identify and develop PCR-based molecular markers closely linked to *cor* that could be used for efficient marker-assisted selection in a broad spectrum of lettuce cultivars. Ideally, such markers would be codominant, easy to score, and inexpensive. We were able to identify several suitable linked markers based on single nucleotide polymorphisms (SNPs, Brookes 1999) that can be analyzed by PCR and without the use of restriction enzymes.

Materials and methods

Plant materials and disease screens

The segregation of resistance and search of *cor* markers was based on the analysis of a F_2 progeny and derived F_3 families ($F_{2:3}$ population) derived from a cross between the resistant crisphead lettuce 'Green Lake' and the butterhead lettuce 'Diana' that lacks any known genes for resistance to CR (GD population). A total of 102 F_3 families were derived from randomly selected F_2 individuals and tested for resistance. Disease screens were carried out by inoculation of one-week-old plants with suspensions of *R. suberifaciens*, isolate CA1, as described previously; plants were maintained in the greenhouse and scored on a nine-point scale for symptoms approximately one month after inoculation (Brown and Michelmore 1988). At least 24 plants were analyzed for each F_3 family. In all screens, 'Green Lake' and 'Diana' were included as resistant and susceptible controls, respectively. Plants showing the same scores as 'Green Lake' and 'Diana' were classified as resistant and susceptible, respectively. Disease screens were carried out several times over a number of years to ensure the correct phenotypic evaluation. Families that showed inconsistencies with marker data in particular were retested to confirm their reaction to corky root.

Molecular techniques

DNA was extracted from leaves of individual F_2 plants or pools of at least 20 individuals from single F_3 families using a modified CTAB (cetyltrimiethylammonium bromide) procedure (Bernatzky and Tanksley 1986). Approximately 2 g tissue/sample were used.

The identification of new markers was simultaneously carried out using two different strategies. The first was based on the analysis in the GD population of restriction fragment length polymorphism (RFLP) markers that were linked in

existing maps to the RFLP *CL658*. The second was based on a bulked segregant analysis (BSA) using the GD population with random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers (Williams et al. 1990; Michelmore et al. 1991; Zietkiewicz et al. 1994). The second approach was followed by the development of PCR-based SNP markers (Syvänen 2001).

To convert RFLPs into PCR-based markers, the corresponding cDNA RFLP probes cloned into pARC7 were sequenced using anchored poly(dT) and poly(dC) primers (Alexander et al. 1984; D. Lavelle, unpublished data). Oligonucleotide (~20mer) primers were designed using OLIGO primer analysis software version 5.0 (Molecular Biology Insights, Cascade, Colo.) and Primer 3 (Rozen and Skaletsky 2000) and were used to amplify the corresponding genomic DNA from both 'Green Lake' and 'Diana'. Approximately 30 ng DNA were used as template in a 20-μL reaction volume that contained 1× PCR buffer (10 mmol Tris–HCl/L (pH 8.3), 50 mmol KCl/L, and 0.01% w/v gelatin), 125 μmol each dNTP/L, 0.5 μmol of each primer/L, 2 mmol $MgCl_2$/L, and 1 U *Taq* polymerase. Amplifications were performed in a PE-9600 thermocycler (PE Applied Biosystems, Foster City, Calif.) starting with an initial denaturation for 2 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 60 °C, and 1 min at 72 °C; and a final extension for 5 min at 72 °C. The amplified fragments were gel purified (Qiaex II Kit, Qiagen, Valencia, Calif.), ligated to pCR 2.1 vector (Invitrogen, Carlsbad, Calif.), and sequenced. Sequencing was carried out on an ABI Prism 377 sequencer (PE Applied Biosystems) using fluorescently labeled di-deoxy terminators (ABI Prism Ready Reaction kits; PE Applied Biosystems).

For the direct analysis of RFLPs on the GD population, Southern blot analyses were conducted according to standard protocols (Sambrook et al. 1989) using Hybond N+ membranes (Amersham Biosciences, Piscataway, N.J.). Probes were labeled with ^{32}P using the random-primer method (MultiPrime, Amersham Biosciences).

For the amplification of RAPD and ISSR markers, approximately 30 ng genomic DNA was used as template in a 20-μL reaction volume that contained 1× PCR buffer (10 mmol Tris–HCl/L (pH 8.3), 50 mmol KCl/L, 0.01% w/v gelatin), 125 μmol each dNTP/L, 0.5 μmol primer/L, 2 mmol $MgCl_2$/L, and 1 U *Taq* polymerase. The amplifications were performed starting with an initial denaturation for 30 s at 94 °C; three cycles of 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C; 32 cycles of 10 s at 94 °C, 30 s at 35 °C, and 1 min at 72 °C; and a final extension for 5 min at 72 °C.

RAPD- and ISSR-PCR fragments linked to *cor* were gel purified, cloned into pCR 2.1, and sequenced as described above. Validation of the cloned fragments was carried out by hybridization to Southern blots of the corresponding RAPD or ISSR gels. Chemiluminescent detection was performed according to the manufacturer's instructions (ECL kit by Amersham). To sequence the cloned PCR fragment in both resistant and susceptible genotypes, two compatible primers of approximately 20 bp were designed internal to the sites where the RAPD or ISSR primers annealed. Amplifications, cloning of PCR bands, and sequencing were carried out as described above for the conversion of RFLPs into PCR markers. When amplification resulted in a single product, the PCR product was sequenced directly.

To design allele-specific primers, the allelic sequences were aligned and any polymorphisms detected. If indel (insertion–deletion) polymorphisms were identified, primers were designed either to match sequences flanking the indel to provide length polymorphism markers or to match sequences within the indel to provide allele-specific primers. For SNPs, allele-specific primers were initially designed with their 3′ end coinciding with the SNP and the rest of the primer matching exactly both alleles as described by Newton et al. (1989). To improve the discrimination of the primers, a base two or three positions from the 3′ end of the primer was changed to force a mismatch in both alleles (Newton et al. 1989; Drenkard et al. 2000). Pairs of primers that produced specific amplifications for one of the alleles were multiplexed with pairs that produced specific amplifications for the other allele; only pairs producing different band sizes were combined. For electrophoresis involving fragments less than 300 bp, a mixture of 0.70% w/v agarose and 1.25% w/v Synergel (Research Products International Corp., Mount Prospect, Ill.) was used.

Genetic analysis

Segregation data were analyzed using the program MAPMAKER (Lander et al. 1987). Loci were grouped using an LOD threshold of 3.0. Map distances were calculated using the Kosambi mapping function (Kosambi 1944).

Results

Of the 102 F_3 families derived from 'Green Lake' × 'Diana' (GD population), 28 were resistant (homozygous, *cor/cor*), 50 segregated approximately 3:1, susceptible to resistant (heterozygous, *Cor/cor*), and 24 were susceptible (homozygous, *Cor/Cor*), consistent with a recessive allele at a single locus determining resistance ($p = 0.5$).

As a first step, we attempted to determine the position of *cor* relative to the RFLP marker *CL658*, which was the closest marker previously found to *cor* (Kesseli et al. 1994). Other RFLP markers linked to *CL658* were also analyzed. These markers had been mapped using cDNA probes and two mapping populations, 'Calmar' × 'Kordaat' (CK population; (Kesseli et al. 1994)) and 'Vanguard 75' × *L. saligna* UC82US1 (VSL population; R. Kesseli and R. Michelmore, unpublished). Neither of these populations segregated for resistance. Initially, we tried to convert the RFLP markers into PCR-based markers that could be used on the GD population. The probe from the RFLP marker *CL658* and the probes for three neighboring RFLP markers in the CK map (*CL201*, *CL635*, and *CL1402*; Fig. 1*c*) were sequenced. Pairs of primers were then designed and the corresponding loci amplified from both 'Green Lake' and 'Diana'. Considering the four loci together, a total of ~1950 bp were sequenced for each cultivar (Table 1). No polymorphisms were identified between 'Green Lake' and 'Diana' for any locus, except between sequences derived from *CL635*; however, the polymorphism identified by primers derived from *CL635* sequences mapped to a difference linkage group and was not considered further.

Fig. 1. Alignment of four local genetic maps of *Lactuca* spp. around the *cor* locus. (*a*) An intra-specific *L. sativa* map from an F_2 population generated by crossing a crisphead cultivar resistant to corky root ('Green Lake') and a susceptible butterhead cultivar ('Diana'). (*b*) An inter-specific map constructed using an F_7 recombinant inbred line population derived from *L. sativa* 'Salinas' × *L. serriola* (Johnson et al. 2000). (*c*) An intra-specific map from an F_2 population generated by crossing *L. sativa* 'Calmar' (crisphead) and *L. sativa* 'Kordaat' (butterhead) (Landry et al. 1987; Kesseli et al. 1994). (*d*) An inter-specific map generated using an F_2 population from *L. sativa* 'Vanguard 75' × *L. saligna* UC82US1 (R. Kesseli and R. Michelmore, unpublished). AFLP markers begin with E, microsatellite markers begin with L or F, RFLPs begin with CL, SCAR markers begin with SC, RAPD markers begin with O. Markers generated using the 'Green Lake' × 'Diana' population that could be transferred to maps are boxed.

Table 1. Molecular description of the alleles at nine loci linked to the *cor* locus in two lettuce cultivars.

	GenBank accession No.		Fragment size (bp)		A+T (%)		No. of SNPs[a]				No. of indels[b]	
Locus	GRN[c]	DIA[c]	GRN	DIA	GRN	DIA	A–G	A–T	G–C	G–T	Single nucleotide	>1 nucleotide
CL 658	AY207409	AY207410	420	420	56	56	0	0	0	0	0	0
CL 201	AY207403	AY207404	246	246	69	69	0	0	0	0	0	0
CL 1402	AY207425	AY207426	814	814	60	60	0	0	0	0	0	0
	AY207427	AY207428										
SCAJ12	AY207421	AY207422	626	626	65	65	1	1	0	1	0	0
SC853	AY207417	AY207418	275	275	61	60	1	0	0	1	0	0
SCY15	AY207399	AY207400	348	348	70	69	0	1	0	1	0	0
SCO07	AY207423	AY207424	480	481	56	56	4	0	1	3	1	0
SC268	AY207405	AY207406	1030	843	59	62	19	1	0	2	1	1
SC074	AY207411	AY207412	1242	1242	66	67	4	2	0	5	2	0

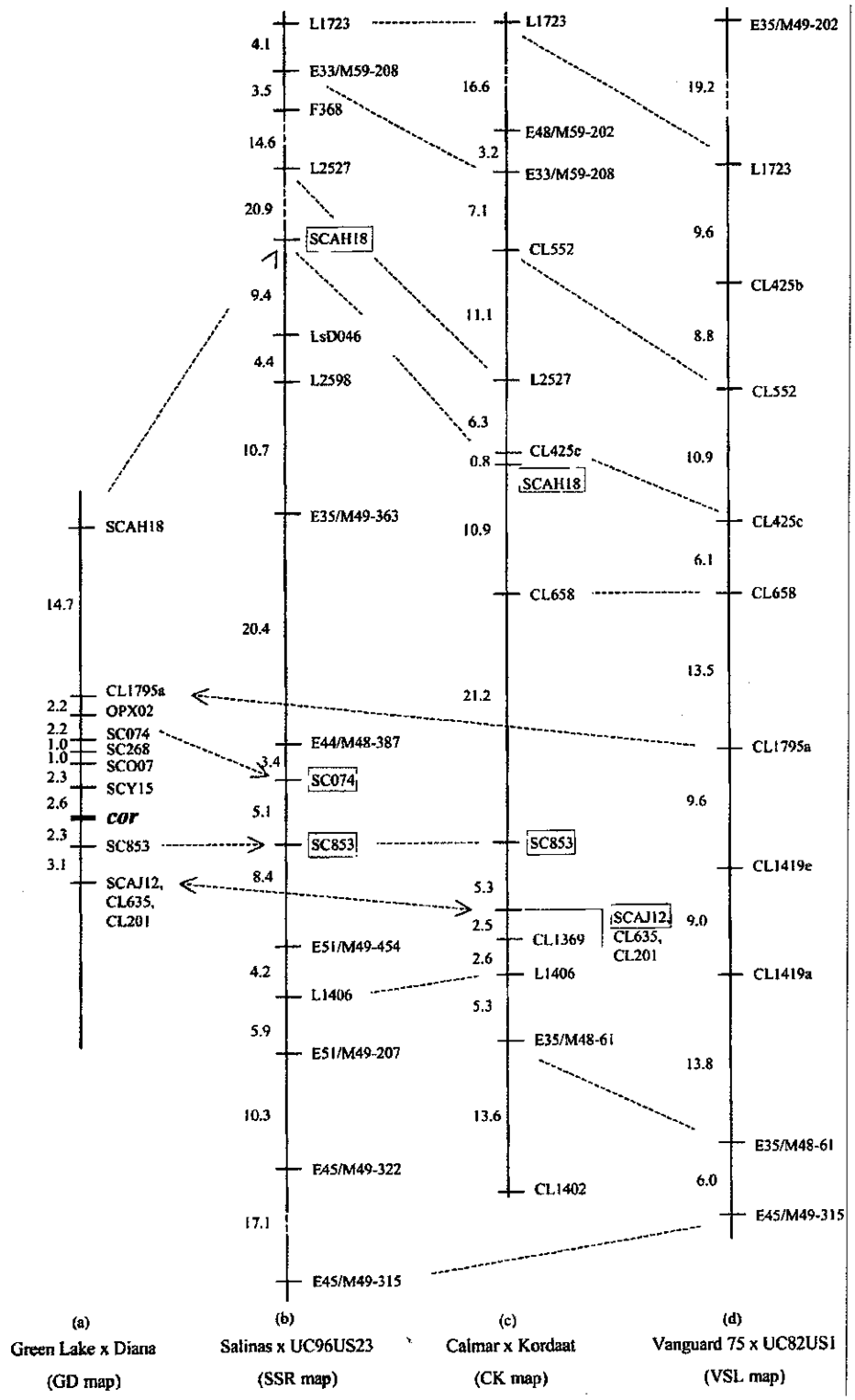
Note: The first three loci were derived from cDNA sequences, the last six loci correspond to non-coding genomic DNA fragments.
[a]Single nucleotide polymorphism.
[b]Insertion–deletions.
[c]GRN, 'Green Lake'; DIA, 'Diana'.

The lack of polymorphism within the regions homologous to the proces required us to carry out conventional RFLP analysis of the GD population with markers linked to *CL658*. DNA from 'Green Lake', 'Diana', and two bulks of DNA from homozygous resistant progeny and homozygous susceptible progeny were cut with eight restriction enzymes (*Eco*RI, *Hin*dIII, *Bam*HI, *Eco*RV, *Bgl*II, *Dra*I, *Xba*I, and *Sst*I) and hybridized to seven RFLP probes: the four mentioned above plus *CL1369*, *CL1419*, and *CL1795*. The last two probes detected RFLPs linked to CL658 in the VSL map (Fig. 1*b*). Three (*CL1795*, *CL201*, and *CL635*) showed polymorphism between parents and between the bulks. These were hybridized to 24 homozygous resistant F_2 individuals from the GD population digested with *Eco*RI for *CL1795* and *CL201* or with *Bgl*II for *CL635* and mapped (Fig. 1*a*). *CL1795a* mapped ~11 cM to one side and *CL201* and *CL635* mapped ~5 cM to the other side of *cor*. None of the RFLP markers were tightly linked to *cor*.

To identify additional and more closely linked markers, BSA was carried out using RAPD and ISSR markers. Two bulks of genomic DNA were prepared, one containing 17 homozygous resistant individuals, the other with 21 homozygous susceptible individuals. A total of 1700 RAPD primers corresponding to the complete sets from Operon Technologies (Alameda, Calif.) and the University of British Columbia (Vancouver, B.C.) were checked. This resulted in the identification of eight dominant markers linked to *cor*: $OPAH18_{1000}$, $OPX02_{1200}$, $OPO07_{2400}$, $OPAJ12_{690}$, $UBC268_{934}$, and $UBC853_{325}$ were in *trans* with resistance, whereas $UBC74_{2000}$ and $OPY15_{440}$ were in *cis* with the resistance allele. None of these markers, however, absolutely cosegregated with *cor*.

The six markers closest to *cor*, $OPO07_{2400}$, $OPAJ12_{690}$, $UBC74_{2000}$, $UBC853_{325}$, $UBC268_{934}$, and $OPY15_{440}$, were converted into codominant SCARs (Fig. 2; Table 2). The allelic sequences for 'Green Lake' and 'Diana' were obtained for each locus. In total, the sequences analyzed accounted for more than 4 kb (Table 1); none contained obvious open reading frames. Overall, there were 11.6 SNPs every 1 kb. Single base indels were 1/10 less frequent than SNPs. The sequences at $UBC268_{934}$ were interesting because the 'Green Lake' allele contained a 178-bp insertion that was absent in 'Diana'. Sequence analysis suggested that this insertion may correspond to an *Ac/Ds*-like element (S. Moreno-Vázquez, unpublished).

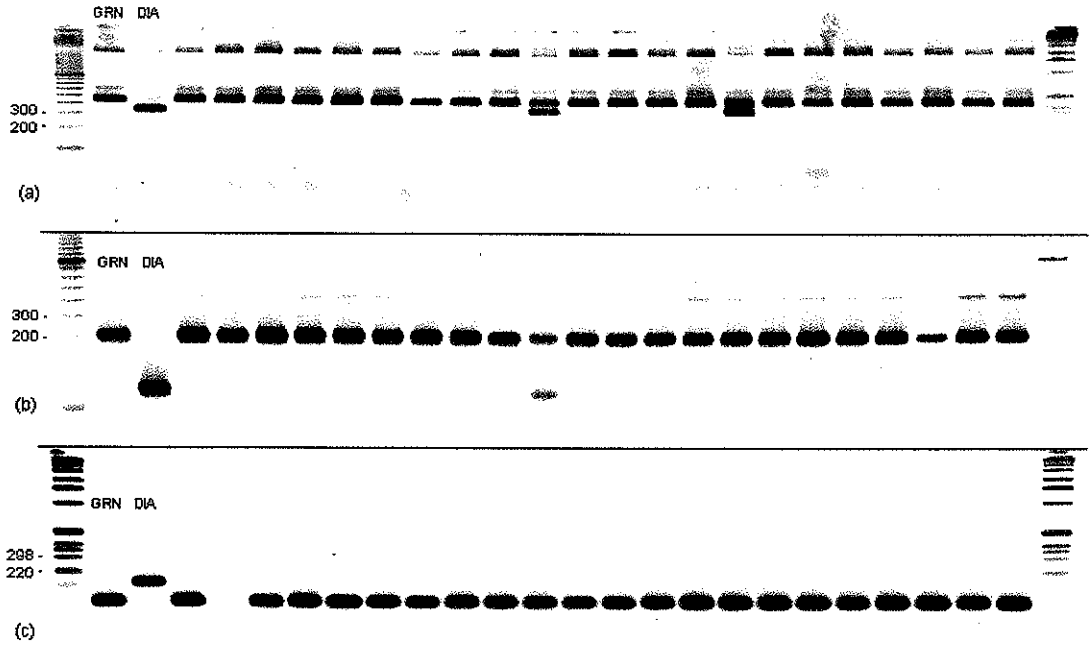
Allele-discriminating primers were designed based on 22 SNPs and the 178-bp insertion. We did not wish to optimize the PCR cycling conditions for each marker, but rather used standard conditions that could be applied to multiple markers and many samples. Therefore, primers were carefully optimized for each marker. Most of these SNP-based markers used allele-specific oligonucleotide primers that had (at least) the most 3′ base matching one allele and mismatching the other. To improve discrimination of these primers, they were normally designed with a sub-terminal base mismatching both alleles. This strategy for primer design could be used even when only very few polymorphisms were available, as occurred with *SCAJ12*, *SC853*, and *SCY15*, where alleles only differed by 3, 2, and 2 point mutations, respectively. SC853 represented the only case where specific prim-



ers without sub-terminal mismatches produced polymorphic bands useful as codominant markers. Over 60% of SNPs were A→G (T→C) (Table 1) and the detection of polymorphisms relied mainly on C:A or G:T mismatches (Table 2). It was relatively straightforward to obtain allele-specific primers when an extra 3′ sub-terminal destabilizing mismatch was added.

However, pairs of allele-specific primers that were informative when used separately often did not produce the desired results when combined. Commonly, only one pair

Fig. 2. PCR markers SC074 (*a*), SCO07 (*b*), and SCY15 (*c*) amplified from parental lines 'Green Lake' (GRN) and 'Diana' (DIA) and from 22 homozygous resistant $F_{2:3}$ families. Two recombinants are observed for SC074 (lines 13 and 18) and one is observed for SCO07 (line 13). See Table 2 for primer sequences and fragment sizes. Numbers on the left show molecular size in base pairs.



provided specific amplification, whereas the other resulted in weak or non-specific amplifications. A variety of primer pairs had to be tested to identify compatible ones. *SCAJ12* was the only marker where separate pairs of primers were discriminatory, but no compatible combination of pairs of primers could be found; it was therefore necessary to amplify each sample twice to identify each allele. It also happened that some combinations of primers detected the corresponding alleles in homozygous genotypes, but not in heterozygous individuals. Using the standard PCR conditions for *SCO07*, the 'Diana' allele did not amplify well in heterozygous templates. A 1/5 reduction in the concentration of the 'Green Lake'-specific primer (O07GRNKF4) permitted a better amplification of both alleles in the heterozygous and also removed non-specific background amplifications. The same problem was found for *SCY15*. In this case, a poly(A) tail in the 5′ end of the 'Green Lake'-specific primer (originally added to provide a length polymorphism; Table 2) dramatically improved the amplification of both 'Green Lake' and 'Diana' alleles in the heterozygous for unclear reasons. Segregation analysis using the SNP-based markers confirmed the lack of a marker that absolutely cosegregated with *cor* (Fig. 1*a*).

A local genetic map around *cor* was constructed using all of the newly developed markers (Fig. 1*a*). Some of the new markers also segregated in other *Lactuca* populations and could be mapped: *SC853* and *SCAJ12* in the CK population (Fig. 1*c*), and *SC853* and *SC074* in the 'Salinas' × *L. serriola* UC96US23 population (SSR population; Fig. 1*b*). UC96US23 is a susceptible *L. serriola* accession; the SSR population is the core mapping population currently used in our laboratory. The order of the markers was consistent in all three maps, permitting location of *Cor*/*cor* on them (Fig. 1). Interestingly, none of these maps are densely populated in the vicinity of *cor* and none of them provided additional candidates for more closely linked markers.

The utility of the new markers as diagnostic for *cor* was evaluated by screening diverse germplasm both for resistance to CR and for molecular marker genotypes. A total of 61 cultivars and 6 landraces were analyzed (Table 3). They included susceptible and resistant genotypes from all of the major lettuce types including crisphead, romaine, butterhead, batavia, latin, and leafy. Normally, resistant cultivars had the "B" ('Green Lake') allele for all markers. Four exceptions were found: PI164939 and PI164937, both leafy types having the "A" ('Diana') allele for *SCAJ12* and *SC268*; 'Alpha 2' and 'Cowboy', both crisphead types having A for *SCAJ12*. All crisphead and butterhead susceptible cultivars except for 'Diana', 'Mildura', 'Mariska', 'Calmar', and 'Amplus' had the haplotype *ABBAAA* (*SCAJ12-SC853-SCY15-SCO07-SC268-SC74*). All romaine susceptible cultivars had the haplotype *BBBABB*, therefore *SCO07* is the only marker available for predicting resistance in romaine cultivars. Without exception, all of the susceptible cultivars had the A allele for at least one of the markers. Interestingly, for the closest markers flanking *cor* (*SC853* and *SCY15*), the B allele was the most common for not only resistant but also susceptible cultivars. The frequency of *SCY15* was the most extreme, being *A* for only three susceptible butterhead cultivars: 'Mariska', 'Diana', and 'Mildura' (one of the parents of 'Diana'). These three cultivars were also *A* for *SC853*. Therefore, although no one marker was absolutely diagnostic for resistance, any two resistant and susceptible accessions could always be distinguished by at least one

Table 2. Characteristics of the six SCAR markers developed around the *cor* locus.

Marker	Allele-specific PCR[a]		Mismatches		Band size (bp)	
	Primer[b]	Primer sequence (5′→3′)	Type	Allele	*Cor Cor*	*cor cor*
SCAJ12[a]	AJ12GRNKF2b	AAC ACA TAC TGG GCG AAC	A:G	DIA	596	105
			G:G	GRN and DIA		
			C:A	DIA		
	AJ12R	TCA GTT CCC GTA TGG TGA TAA				
	AJ12F2	TCT ACC CAA GCA TCG TGT				
	AJ12DIAKR22	TCG CCG AGT AGT CGA CCA TA	A:G	GRN and DIA		
			A:C	GRN		
SC853	853DIAF1	TAG TAG CAA AGA AGA GAG	G:A	GRN	192	141
	853GRNF2	GGT TTT CGC CAT TAC TTT	T:G	DIA		
	853R4	CTA GAA AAC TGA GAT GAG				
SCY15	Y15F1	AGC GTT ATA TCT CTC CTC TC			160	94
	Y15GRNPAKR1	$(A)_{20}$ TGT GTA GTA CTC CTC ATA GAT	G:G	GRN and DIA		
			T:C	DIA		
	Y15DIAKR2	ATA TTC CAG ACA AGT GAT TA	A:A	GRN and DIA		
			A:A	GRN		
SCO07	O07GRNKF4	AGA GTT GAC AGA GCA ACA CG	A:A	GRN and DIA	134	284
			G:T	DIA		
	O07DIAKF7	ATG GGC TAA AAC ACT CAC AG	C:T	GRN and DIA		
			G:G	GRN		
	O07R	ATG TGG CTA TGA CTT CAG A				
SC268[b]	268GRNF3	GAG CTC GAG CTT GGT TAG G	InDel	DIA	262	>506
	268DIAF4	TGA TGT TGC ATC AAA AAT T	T:G	GRN		
			T:G	GRN		
	268GRNR2	CTC TCG CTG TGC TGT C	T:G	DIA		
			C:A	DIA		
	268R2	ACC TGT GAT TTT GAG TTT GC				
SC074[b]	74F1				315	418
	74DIAR7	ACA ACT AAC AAA AAA GGG AG	A:C	GRN		
			A:C	GRN		
			G:A	GRN		
			G:T	GRN		
	74GRNKF13	GTC CCA TTG CTC TCA GCG	G:G	GRN and DIA		
			G:A	DIA		
	74R2	ATT CCT TGA GAT TCC ATA GT				

Note: The nucleotides mismatching one of the alleles (usually the 3′ terminal nucleotide) or both alleles (sub-terminal non-specific mispriming sites) appear in bold in the primer sequences. In the fourth column, for each primer the mismatch types are specified starting above with the most 5′ mismatch. Each mismatch is described by two letters separated by the colon mark, the first letter refers to the primer base, the second to the mismatching base in the template sequence. In the fifth column, the alleles affected by the mismatch are cited. The sixth and seventh columns indicate the size of the alternative alleles for each molecular marker.

[a]Two PCRs are required per sample. One with primers AJ12GRNKF2b and AJ12R and a second with primers AJ12F2 and AJ12DIAKR22.

[b]Additional bands of high molecular weight may also be amplified.

marker. Null alleles were only present for marker *SCY15* in landraces PI171675 and PI171676. Markers were seldom heterozygous for landraces and never for modern cultivars.

Discussion

As expected for a monogenic character in an $F_{2:3}$ population, resistance to CR segregated close to 1:2:1 in the population derived from 'Green Lake' and 'Diana'. This crisphead × butterhead population permitted the identification of RFLP and PCR-based markers linked to the recessive resistance gene *cor* (Fig. 1). The absence of polymorphism within regions homologous to the cDNA probes in 'Green Lake' and 'Diana' prevented the transformation of RFLPs into PCR-based markers and their use for germplasm screens and marker-assisted selection. Only three RFLP markers could be transferred to the local GD map. The remainder of the markers that we identified closely linked to cor were PCR-based markers found using bulked segregant analysis.

Relative to other studies, the search of molecular markers by BSA linked to *cor* was inefficient. A total of 1700 RAPD and ISSR primers were used and over 10 000 fragments assayed, but the closest marker found was 2.3 cM away from the resistance gene (Fig. 1). For tagging *Dm8* (a gene for resistance to *Bremia lactucae*) and *plr* (a gene for resistance to *Plasmopara lactucae–radicis*) in lettuce, relatively low numbers of primers were analyzed and several markers tightly linked to these resistance genes were obtained (Kesseli et al. 1993; Witsenboer et al. 1995). There are many examples in other plant species where a moderate number of primers (be-

Table 3. Evaluation of markers linked to *cor* in different types of resistant and susceptible lettuce cultivars.

		SCAJ12	*SC853*	*cor*[a]	*SCY15*	*SCO07*	*SC268*	*SC074*
Distance between loci (cM):			3.1	2.3	2.6	2.3	1.0	1.0
Cultivar	Leaf or head type							
'Diana'	Butterhead	A[b]	A	S	A	A	A	A
'Mildura'	Butterhead	A	A	S	A	A	A	A
R4T47	Genetic stock	A	A	S	A	A	A	A
'Mariska'	Butterhead	A	A	S	A	A	B	A
PI171675	Leaf	B	A	S	Null allele	B	A	B
PI171676	Leaf	B	A	S	Null allele	B	A	B
'Calmar'	Crisphead	B	A	S	B	A	A	A
'Lollo Rosa'	Leaf	B	A	S	B	A	B	B
'Babystar'	Romaine	B	A	S	B	A	B	B
'Oak Leaf 79'	Oak leaf	A	B	S	B	A	A	A
'Avondefiance'	Butterhead	A	B	S	B	A	A	A
'Butter Crunch'	Butterhead	A	B	S	B	A	A	A
'Captain'	Butterhead	A	B	S	B	A	A	A
'Kordaat'	Butterhead	A	B	S	B	A	A	A
'Passion Blonde'	Butterhead	A	B	S	B	A	A	A
'Saffier'	Butterhead	A	B	S	B	A	A	A
'Alpha'	Crisphead	A	B	S	B	A	A	A
'Avoncrisp'	Crisphead	A	B	S	B	A	A	A
'Bullseye'	Crisphead	A	B	S	B	A	A	A
'Fulton'	Crisphead	A	B	S	B	A	A	A
'Mesa'	Crisphead	A	B	S	B	A	A	A
'Oswego'	Crisphead	A	B	S	B	A	A	A
'Salinas'	Crisphead	A	B	S	B	A	A	A
'Salinas 88'	Crisphead	A	B	S	B	A	A	A
'Target'	Crisphead	A	B	S	B	A	A	A
'Vanguard 75'	Crisphead	A	B	S	B	A	A	A
'Calgary'	Crisphead	A	B	S	B	A	A	A
'Diamond'	Crisphead	A	B	S	B	A	A	A
'Fortunas'	Crisphead	A	B	S	B	A	A	A
'Silverado'	Crisphead	A	B	S	B	A	A	A
'Talia'	Crisphead	A	B	S	B	A	A	A
'Gallega'	Latin	A	B	S	B	A	A	A
'Amplus'	Butterhead	A	B	S	B	A	A	B
'Valorix'	Oak leaf	A	B	S	B	B	A	A
PI164939	Leaf	A	B	R	B	B	A	B
PI164937	Leaf	A	B	R	B	B	A	H
'Alpha2'	Crisphead	A	B	R	B	B	B	B
'Cowboy'	Crisphead	A	B	R	B	B	B	B
'Conchita'	Romaine	B	B	S	B	A	A	A
'Alisia'	Romaine	B	B	S	B	A	B	B
'Camino Real'	Romaine	B	B	S	B	A	B	B
'Pinokkio'	Romaine	B	B	S	B	A	B	B
'Tiberius'	Romaine	B	B	S	B	A	B	B
'Darkland Cos'	Romaine	B	B	S	B	A	B	B
'Green Towers'	Romaine	B	B	S	B	A	B	B
'Valmaine'	Romaine	B	B	S	B	A	B	B
PI171665	Romaine	B	B	S	B–H	A	H	B
'Fayal'	Batavia	B	B	S	B	B	A	A
'Florida Buttercrisp'	Butterhead	B	B	R	B	B	B	B
'Big Ben'	Crisphead	B	B	R	B	B	B	B
'Bronco'	Crisphead	B	B	R	B	B	B	B
'Green Lake'	Crisphead	B	B	R	B	B	B	B
'Marquette'	Crisphead	B	B	R	B	B	B	B
'Misty Day'	Crisphead	B	B	R	B	B	B	B

Table 3. (*concluded*).

		SCAJ12	*SC853*	*cor*[a]	*SCY15*	*SCO07*	*SC268*	*SC074*
Distance between loci (cM):		3.1	2.3	2.6	2.3	1.0	1.0	
'Montello'	Crisphead	B	B	R	B	B	B	B
'Raleigh'	Crisphead	B	B	R	B	B	B	B
'Tracer'	Crisphead	B	B	R	B	B	B	B
'South Bay'	Crisphead	B	B	R	B	B	B	B
PI171669	Leaf	B	B	R	B–H	B	B	B
'Clemente'	Romaine	B	B	R	B	B	B	B
'Tall Guzmaine'	Romaine	B	B	R	B	B	B	B
'Bacio'	Romaine	B	B	R	B	B	B	B
'Coastal Star'	Romaine	B	B	R	B	B	B	B
'Conquistador'	Romaine	B	B	R	B	B	B	B
'Entrada'	Romaine	B	B	R	B	B	B	B
'Gladiator'	Romaine	B	B	R	B	B	B	B
'Mardi Gras'	Romaine	B	B	R	B	B	B	B
'Medaillon'	Romaine	B	B	R	B	B	B	B
'Nader'	Romaine	B	B	R	B	B	B	B

Note: Cultivars are classified into groups separated by a horizontal line according to the molecular haplotype. The two cultivars used to construct the genetic map are in bold.

[a]S, susceptible to corky root rot; R, resistant to corky root rot.

[b]A, homozygous with 'Diana' allele; B, homozygous with 'Green Lake' allele; H, heterozygous or segregating.

tween 150 and 300) resulted in the identification of markers cosegregating with the target gene (Haley et al. 1993; Miklas et al. 1993; Johnson et al. 1995).

The efficiency of the different types of 3′ terminal mismatches for allele-specific amplification varies (Kwok et al. 1990; Brookes 1999); A:G, C:C, and A:A are considered strong mismatches that aid discrimination between alleles; G:G are medium mismatches; and C:A and G:T are considered stable mismatches and consequently undesirable for allelic discrimination. However, such mismatches are the basis of most SNP analysis in humans because of the over abundance (67%) of the transition type A→G (T→C) (Brookes 1999; Prince et al. 2001). In our study, A→G (T→C) was also the most frequent type of SNP and the detection of polymorphisms was mainly based on C:A or G:T mismatches. Even for these stable terminal mismatches, we found it relatively straightforward to obtain allele-specific primers with the additional 3′ sub-terminal destabilizing mismatch.

The alignment of markers around *cor* in the four separate genetic maps (Fig. 1) indicated a good conservation of the marker order, even when different *Lactuca* species were considered. This supported synteny between the markers. In all four maps, *cor* was located in areas with a low density of markers. In the VSL map, the markers E35/M49-202 and E45/M49-315 defined a broad interval encompassing the *Cor*/*cor* locus. The same two markers are present in a recently published dense AFLP map derived from an interspecific *Lactuca* cross (Jeuken et al. 2001); again, this region has a low density of markers relative to other areas of the genome. This is consistent with our difficulties in finding markers tightly linked to *cor*. Two causes may be responsible for the rarity of markers: absence of polymorphism around *cor* and (or) an elevated rate of recombination in the area. When comparing the sequences from 'Green Lake' and 'Diana' for the six non-coding loci closest to *cor*, a ratio of 11.6 SNPs for every 1000 bp was found. This suggests that the absence of polymorphism is probably not the major cause of the paucity of markers. The paucity of markers in this region in all three maps derived from very different parents is consistent with high rates of recombination around *cor*.

The *cor* gene does not map to any of the known clusters of resistance genes. There are at least three major clusters of disease resistance genes known in lettuce (Kesseli et al. 1994). However, these mainly contain dominant genes for resistance to foliar diseases, particularly downy mildew.

Commonly, the utility of molecular markers for marker-aided selection is a function of their proximity to the gene being indirectly selected (Haley et al. 1993). The markers identified in this paper will be useful for marker-aided selection in populations derived from parents with known haplotypes. However, analysis of the SCAR markers for *cor* on a large collection of cultivars demonstrated that the closest markers (*SC853* and *SCY15*) were the least correlated with resistance and were therefore the least diagnostic for *cor*. There are several examples of markers tightly linked to resistance genes, but whose use present problems in other materials different from the original lines in which they were identified (Lawson et al. 1998). The most common scenario is when susceptible germplasm has the marker correlated with resistance. This has been observed many times in *Phaseolus* when the introgressed gene and the genetic background share common origins, for instance, resistance introgressed from Mesoamerican germplasm into Mesoamerican cultivars. Resistant materials having the marker correlated with susceptibility has been less frequently observed (Haley et al. 1993; Miklas et al. 1993; Geffroy et al. 1998; Miklas et al. 2000). The pedigrees of the lettuce germplasm surveyed provide no obvious explanation for the observed molecular haplotypes around *cor*. Although some of the markers linked

to *cor* showed anomalous associations with resistance, the overall haplotypes allow diagnosis of resistance to CR in cultivated germplasm.

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Corky Root of Lettuce Caused by Strains of a Gram-Negative Bacterium from Muck Soils of Florida, New York, and Wisconsin

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Slow-growing bacteria similar to the bacterium causing lettuce corky root (CR) in California (strain CA1) were isolated from muck soils of Florida, New York, and Wisconsin, using lettuce seedlings as bait. All strains were tested for reaction with polyclonal antibodies produced against strain CA1 and for pathogenicity on CR-susceptible (Salinas) and CR-resistant (Green Lake) lettuce cultivars in a greenhouse. Five strains from Florida, three from New York, and three from Wisconsin induced severe CR symptoms on Salinas and mild symptoms on Green Lake. All strains were gram-negative, aerobic, oxidase positive, and catalase positive and reduced nitrate to ammonia. Whole-cell fatty acid compositions were similar for all strains and resembled that of *Pseudomonas paucimobilis*. Since this fatty acid pattern is unique, it is suggested that CR of lettuce is caused by strains of the same bacterium in Florida, New York, Wisconsin, and California.

Corky root (CR) of lettuce has been reported in California (20), Florida (12, 13), New York (14, 15), Ontario (6), Wisconsin (1, 21), and Italy (7, 8). The symptoms initially consist of yellow bands on young roots and develop later into dark greenish brown and corked areas covering most of the taproot and main laterals. Infected roots become brittle and break off easily (23). The etiology of this disease was controversial for many years. In the 1960s and 1970s, CR symptoms were attributed to phytotoxic components, such as ammonia (11, 15, 17) or toxins liberated from decomposing lettuce debris (2, 7, 8, 14). Before that time, similar symptoms had been attributed to biotic agents, namely, *Botrytis cinerea* (16), a *Pythium* sp. (C. I. Hannon, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1955.), *Pseudomonas rhizoctonia* (22), and *Xanthomonas vitians* (4). However, Koch's postulates were not fulfilled until recently, when it was shown that a slow-growing, gram-negative bacterium caused CR of lettuce in California (23).

This bacterium has not been reported as the pathogen causing CR in areas outside California. Toxic substances from decomposing lettuce debris were suspected to be the cause of CR in Ontario (L. V. Busch and J. A. Carpenter, Can. Phytopathol. Soc. Proc. 31:23, 1964), Wisconsin (2), New York (J. P. Hartnett and J. W. Lorbeer, Phytopathology 58:1053, 1968), and Italy (7, 8). However, a bacterial causal agent could not be excluded in those areas, because experiments involving decomposing lettuce debris were performed under field conditions or in the greenhouse with field soil for decomposition of lettuce residue. In Wisconsin, a secondary amine was purified from lettuce debris which caused root necrosis of lettuce seedlings (2) but not the typical corkyness. Autoclaved crude extract, however, did cause typical CR symptoms, indicating that the toxic substance was heat stable (2). This toxic substance could have been produced by a bacterium similar to that causing lettuce CR in California, because a heat-stable toxin was isolated from culture filtrate of the CR bacterium (J. Kao, D. H. Mitten, and C. A. Milich, Phytopathology 76:844, 1986).

The objectives of the research reported here were to demonstrate the presence of a gram-negative bacterium in CR-prone soil from Florida, New York, and Wisconsin similar to the bacterium causing lettuce CR in California and to compare various strains for virulence on resistant and susceptible lettuce cultivars and for culture, physiological, and chemical characteristics.

MATERIALS AND METHODS

Soil samples. Samples of corked lettuce roots and attached soil were collected in June 1987 from Oswego County, New York. Soil samples from Florida and Wisconsin were provided by Victor Guzman and Luis Sequeira in May and September 1987, respectively. All samples originated from muck soils in which CR of lettuce had been a problem in previous years.

Isolation procedures. Isolations from soil were made with 2- to 3-week-old lettuce seedlings, cultivar Salinas, as bait. Soil suspensions were made by mixing 50 g of soil from each location in 75 ml of distilled water plus 3 drops of Tween 20. The suspensions were stirred for 10 to 15 min and filtered through six layers of cheesecloth. Suspension (5 ml) was dispensed at the stem base of each of five 2- to 3-week-old Salinas lettuce seedlings in a greenhouse. At 3 to 4 weeks after inoculation, the plants were uprooted and isolations were made from yellow or corked areas on the roots as described previously (23) but without surface sterilization with 0.5% sodium hypochlorite. Slow-growing colonies that appeared similar to those of CR bacteria from California were transferred to S medium (23).

Antibody tests. To distinguish potential CR strains from other slow-growing bacteria, all isolates with colonies similar to those of the CR bacterium were tested for their reaction with polyclonal antibodies. The antibodies were produced by immunizing New Zealand White rabbits with intact and sonicated cells of the first strain of the CR bacterium from California (strain CA1) (23). Serum was precipitated in 40% ammonium sulfate, dialyzed against phosphate-buffered saline (0.01 M phosphate, pH 7.6, 0.14 M NaCl), and purified in a DEAE-cellulose column.

Cultures (5 days old) of the CR strains in S broth were sonicated for 30 to 45 s with a Microson ultrasonic cell disruptor model MS-50 equipped with a CM-1 convertor at 80% power to break up clumps (Heat Systems Electronics,

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Inc., Farmingdale, N.Y.). The concentration of bacteria in suspension was assessed with a spectrophotometer at 650 nm (Spec 20; Bausch & Lomb, Inc., Rochester, N.Y.). Eight dilutions were prepared of each strain in Tris-buffered saline (TBS) (10 mM Tris hydrochloride, 150 mM NaCl, pH 8.0) and applied onto a nitrocellulose membrane with a vacuum manifold (Hybri-dot manifold; Bethesda Research Laboratories, Gaithersburg, Md.). The dilutions amounted to 2×10^8, 5×10^7, 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3, and 1×10^2 cells per dot. *Rhizobium meliloti* (8D15; C. I. Kado, University of California at Davis) was applied in the same amounts as a negative control. The blot was air dried for at least 4 h and then dried at 50°C for 1 h. The blot was soaked in TBST (TBS plus 0.05% Tween 20) and incubated in blocking solution (TBST plus 1% bovine serum albumin, fraction 5) at 37°C with shaking. After 30 min, the blot was rinsed in TBST and incubated in a 1:100-diluted anti-CR strain CA1 immunoglobulin G solution in TBST for 3 to 4 h at 37°C with shaking. All nonreacted antibody was rinsed away by three successive 7-min washes with shaking in an excess of TBST. Anti-CA1 was detected by incubating the blot for 2 h at 37°C with a 1:1,000 dilution of protein A-alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, Mo.). The blot was rinsed in TBST as before. Protein A-alkaline phosphatase conjugate was detected by a color reaction with Nitro Blue Tetrazolium (0.66% [vol/vol] of a 50-mg/ml solution) and 5-bromo-4-chloro-3-indolyl phosphate (0.33% [vol/vol] of a 50-mg/ml solution) in AP buffer (100 mM Tris chloride, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5). Reaction of each CR bacterium strain with the antibody was rated subjectively (0 to 6 scale) by comparison with the reaction of CA1 and the negative control.

Pathogenicity tests. Preliminary pathogenicity tests were done with all strains that reacted with the antibodies and had a colony morphology and growth rate similar to those of the CR bacterium from California (23). Sterile distilled water (10 ml) was pipetted onto plates with 7- to 10-day-old pure cultures, and 2 to 3 ml of suspension was dispensed at the stem bases of two or three 2-week-old Salinas lettuce seedlings for each strain.

Extensive pathogenicity tests to fulfill Koch's postulates were performed in a greenhouse with 12 strains that caused symptoms in preliminary tests, as described previously for a California strain of the CR bacterium (CA1) (23). Inocula were prepared by centrifuging 5-day-old cultures in S medium broth for 20 min at 9,150 × *g* and suspending the pellets in the same amount of distilled water. Bacterial concentrations were estimated with a spectrophotometer and were adjusted to $(1.7 \pm 0.7) \times 10^9$ CFU/ml. Lettuce seedlings (2 weeks old) in vermiculite were inoculated by dispensing 3 ml of bacterial suspension or distilled water (controls) at the stem bases of each plant. Plants of each treatment were placed on saucers in insect-proof cages 50 cm apart to avoid cross contamination by fungus gnats and water splashing. There were six plants per treatment. The plants were watered alternately with 20 to 25 ml of distilled water, half-strength Hoagland solution, or 0.005 M $Ca(NO_3)_2$ + 0.005 M KNO_3. Minimum and maximum temperatures were 16.8 ± 2.1 and 27.9 ± 1.5°C, respectively. Daylight was extended to 14 h by fluorescent tubes. Four weeks after inoculation, the plants were uprooted and scored for CR severity on a 0 to 9 scale, based on percentages of the taproot infected (5). Isolations were made from three inoculated and control plants as described previously (23), and reisolated colonies were again tested for reaction with polyclonal antibodies and pathogenicity on lettuce seedlings. Three plants were dried in a forced-air drying oven at 80°C for 2 days to determine shoot and root dry weights. CR severity scores and shoot and root dry weights were analyzed by analysis of variance, and residual values were tested for normality (SAS Institute, Inc., Cary, N.C.). To rank the strains, we performed Duncan's multiple-range test.

TABLE 1. CR severity (0 to 9 scale), reisolation of CR bacteria, and dry weights of shoots and roots of lettuce cultivar Salinas 27 days after inoculation with 12 strains of the CR bacterium

Strain	Avg CR score[a]	Reisolation of CR bacteria[b]	Dry wt of shoot (g)[c]	Dry wt of root (g)[c]
None	0.0 ± 0.0 a	0	0.82 ± 0.10 a	0.16 ± 0.03 a
CA1	5.5 ± 0.5 b	2	0.52 ± 0.01 b	0.12 ± 0.03 ab
FL1	7.0 ± 0.0 de	3	0.46 ± 0.04 bc	0.09 ± 0.02 b
FL2	6.0 ± 0.9 bc	3	0.54 ± 0.02 b	0.13 ± 0.04 ab
FL3	5.7 ± 0.8 b	3	0.53 ± 0.02 b	0.13 ± 0.02 ab
FL4	8.2 ± 0.4 f	3	0.17 ± 0.04 d	0.02 ± 0.01 c
FL5	8.2 ± 0.4 f	3	0.14 ± 0.04 d	0.02 ± 0.01 c
NY10	6.8 ± 0.4 d	3	0.44 ± 0.10 bc	0.10 ± 0.03 b
NY11	6.5 ± 0.5 cd	3	0.47 ± 0.02 bc	0.13 ± 0.04 ab
NY12	7.5 ± 0.5 e	3	0.38 ± 0.12 c	0.12 ± 0.03 ab
WI2	7.5 ± 0.5 e	3	0.19 ± 0.04 d	0.04 ± 0.02 c
WI3	8.3 ± 0.5 f	3	0.10 ± 0.04 d	0.02 ± 0.01 c
WI4	7.0 ± 0.0 de	1	0.49 ± 0.02 bc	0.03 ± 0.01 c

[a] Average of six plants. Means followed by different letters differ significantly ($\alpha = 0.05$) according to Duncan's multiple-range test.
[b] Number of plants out of three from which CR bacteria were reisolated.
[c] Average of three plants. Means followed by different letters differ significantly ($\alpha = 0.05$) according to Duncan's multiple-range test.

In a factorial experiment, the same 12 strains were tested for virulence on cultivars Salinas and Green Lake, susceptible and resistant, respectively, to strain CA1 (5). The same procedures were used as described above to grow, inoculate, and evaluate the plants. The concentration of the inoculum was $(7 \pm 2) \times 10^8$ CFU/ml. Minimum and maximum temperatures in the greenhouse were 18.1 ± 1.9 and 28.7 ± 2.4°C, respectively. Daylight was extended to 14 h by fluorescent tubes. Three weeks after inoculation, CR severity scores and shoot and root dry weights were determined. The data were analyzed by analysis of variance including an interaction term (cultivar × strain), and residual values were tested for normality (SAS Institute). To rank the strains, we performed Duncan's multiple-range test for each cultivar separately.

Preliminary identification. (i) Strains of CR bacteria. The following CR strains were included in various physiological and chemical tests: CA1, FL1, FL2, FL3, FL4, FL5, NY10, NY11, NY12, WI2, WI3, and WI4 (Table 1). All strains were grown in S medium broth for 4 days at room temperature with shaking.

(ii) Gram reaction. All strains that caused CR on lettuce seedlings were stained according to the Hucker modification of the Gram-stain procedure (10). *Clavibacter rathayi* (NCPPB 2980) and *Pseudomonas fluorescens* (ATCC 13525; biotype A) served as gram-positive and gram-negative controls, respectively. The CR strains were also subjected to the KOH stringiness test (9), with *P. fluorescens* as the gram-negative control.

(iii) Aerobiosis. All CR strains were incubated for 3 weeks at room temperature on solid S medium under anaerobic conditions (Oxoid jar plus gas-generating kit; Oxoid Ltd., Basingstoke, Hampshire, England). After 3 weeks, the

plates were incubated under aerobic conditions at 27°C for 1 week and checked for growth.

(iv) Oxidase test. Oxidase activity was tested by the method of Kovacs (10), using 4-day-old cultures of 12 strains of the CR bacterium in S broth and 1-week-old cultures on solid S medium. Cultures (1 day old) of *P. fluorescens* and *Pseudomonas syringae* pv. *syringae* (5D4214 from C. I. Kado) on S medium were positive and negative controls, respectively.

(v) Catalase test. Catalase production was determined as described before (10). To rule out the possibility of a weak catalase reaction going unnoticed, active liquid cultures (about 3×10^9 CFU/ml) were mixed 1:1 (vol/vol) with 3% hydrogen peroxide and the production of oxygen was monitored over 10 min at 1-min intervals with an oxygen-sensitive electrode (P5 oxygen sensor; Jensen Instruments, Tacoma, Wash.) (3). Cultures (2 days old) of *P. fluorescens* and *Streptococcus lactis* (ATCC 19435) on S medium served as positive and negative controls, respectively. Oxygen evolution of the bacterial cultures was compared by analyzing intercepts and slopes of individual linear regressions on time by analysis of variance combined with contrast analysis and Duncan's multiple-range test (SAS Institute). For *P. fluorescens*, oxygen production was measured for 30 to 50 s at 5-s intervals until the percent oxygen started to level off. Regression on time was only performed over the initial, linear part of the curve.

(vi) Nitrate reduction. Reduction of nitrate to nitrite was determined by mixing 1 drop of 4-day-old cultures of 12 CR strains in S broth with 2 to 3 drops of 12 N H_2SO_4 and 2 drops of starch iodide solution [as in reference 9, but $Z(I)_2$ was used instead of KI]. Reduction to ammonia was detected by mixing 1 drop of 4-day-old CR culture with 1 drop of Nessler reagent (10). Production of nitrogen gas was monitored by incubating a liquid culture in a culture tube with a submerged gas-trap tube. *Escherichia coli* (DH1 from C. I. Kado) served as a positive control for the conversion of nitrate to nitrite, *P. fluorescens* served as a positive control for reduction to ammonia, and *Pseudomonas solanacearum* was the positive control for denitrification to N_2.

(vii) Fatty acid profiles. Whole-cell fatty acid profiles were compared for all 12 CR strains. Cultures were grown for 4 to 5 days in S broth on a shaker at 28°C. Fatty acids were extracted and methylated by the procedure of Moore et al. (18). Fatty acid methyl esters were separated and analyzed by gas chromatography-mass spectrometry at the Facility for Advanced Instrumentation, University of California at Davis, on a 30-m DB-225 capillary column (J. and W. Scientific, Folsom, Calif.). Column temperatures were raised from 70 to 250°C at 60°C/min. Peaks were analyzed by retention times and by chemical ionization on a mass spectrophotometer (Trio-2 gas chromatograph-mass spectrometer; VG Masslab, Altrincham, United Kingdom).

RESULTS

Isolations. Isolations from soil with lettuce seedlings as bait resulted in 11 strains from Florida, 5 from New York, and 3 from Wisconsin. Eleven strains (FL1, FL2, FL3, FL4, FL5, NY10, NY11, NY12, WI2, WI3, and WI4) were retained for pathogenicity tests and characterization. The colony color of most strains was creamy white when observed from above and yellowish when observed against fluorescent lights. One strain (WI4) was dull yellow, producing a brown diffusible pigment on S medium. On solid S medium, CR colonies were relatively compact and dislodged as intact colonies from the agar plate with a transfer loop. A detailed description of colony morphology of strain CA1 was given previously (23). Colonies of New York strains (NY10, NY11, and NY12), one Wisconsin strain (WI2), and one Florida strain (FL3) were similar to strain CA1: dry, compact, and wrinkled on solid S medium. The other CR strains were slightly more mucoid.

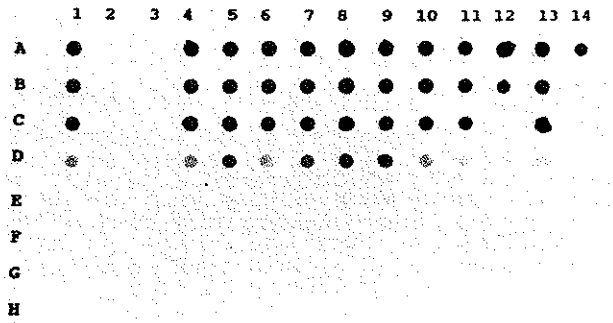

FIG. 1. Reaction of 12 strains of the CR bacterium and *R. meliloti* at eight dilutions with polyclonal antibodies produced against CR bacterium strain CA1, using alkaline phosphatase on an indirect immunoblot. A to H, 2×10^8, 5×10^7, 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3, and 1×10^2 cells per dot, respectively. 1 to 14, CA1, *R. meliloti*, broth control, FL1, FL2, FL3, FL4, FL5, NY10, NY11, NY12, WI2, WI3, and WI4, respectively.

Antibody tests. All CR strains reacted with polyclonal antibodies produced against strain CA1 (Fig. 1). Strain CA1 produced a weak positive reaction at concentrations down to 10^3 cells per dot on nitrocellulose membrane. *R. meliloti*, the negative control, showed a very weak reaction at 2×10^8 cells per dot. The Florida and New York strains and one Wisconsin strain (WI3) reacted at the same dilutions as strain CA1. The Wisconsin strains WI2 and WI4 reacted only at concentrations of 10^6 or more cells per dot.

Pathogenicity tests. All strains that caused CR in preliminary tests were also pathogenic in the detailed pathogenicity tests conducted to fulfill Koch's postulates (Table 1). CR bacteria were reisolated from most (92%) of the roots infected with CR. Noninoculated plants remained healthy, and CR bacteria were not isolated from their roots. Analysis of variance showed that there were significant treatment effects for all dependent variables. The residual values were normally distributed. Duncan's multiple-range test indicated that the five most aggressive strains were FL4, FL5, NY12, WI2, and WI3. These strains initially induced brown necrotic lesions on the taproot rather than the usual yellow lesions followed by corkiness. Shoot and root dry weight were significantly reduced by CR (Table 1). Ranking of the strains based on shoot and root dry weight was similar to that based on CR score (with the exception of WI4).

Comparison of disease reaction of resistant (Green Lake) and susceptible (Salinas) cultivars to the same strains resulted in a significant interaction between strains and cultivars ($F^{12,130} = 4.5$; $P < 0.01$). However, the ranking of strains on these cultivars differed only slightly (Table 2). The five most aggressive strains (FL4, NY10, NY12, WI2, and WI3) caused the same amount of CR on each cultivar, and for each strain, the average CR score was higher on Salinas than on Green Lake. The average CR scores were generally lower than in the previous experiment, probably owing to

TABLE 2. CR severity (0 to 9 scale), reisolation of CR bacteria, and dry weights of shoots and roots of lettuce cultivars Salinas and Green Lake 21 days after inoculation with 12 strains of the CR bacterium

Cultivar	Strain	Avg CR score[a]	Reisolation of CR bacteria[b]	Dry wt of shoot (g)[c]	Dry wt of root (mg)[c]
Salinas	None	0.1 ± 0.4[d]a	1	0.12 ± 0.01 abcd	16.0 ± 1.6 abc
	CA1	2.5 ± 1.7 bcd	3	0.15 ± 0.06 a	21.7 ± 9.9 ab
	FL1	1.7 ± 0.8 b	3	0.14 ± 0.01 ab	24.7 ± 0.5 a
	FL2	1.3 ± 0.5 b	3	0.13 ± 0.02 abc	17.0 ± 5.4 abc
	FL3	1.5 ± 0.6 b	1	0.13 ± 0.03 abc	18.7 ± 2.9 abc
	FL4	4.7 ± 0.8 f	3	0.10 ± 0.01 bcd	16.3 ± 1.2 abc
	FL5	3.2 ± 0.8 cde	3	0.09 ± 0.02 cd	16.3 ± 2.6 abc
	NY10	3.3 ± 1.4 de	3	0.13 ± 0.01 abc	19.0 ± 5.0 abc
	NY11	1.5 ± 1.0 b	3	0.10 ± 0.01 bcd	11.7 ± 2.9 bc
	NY12	4.7 ± 0.8 f	3	0.13 ± 0.01 abc	19.3 ± 1.2 abc
	WI2	3.5 ± 0.6 de	3	0.10 ± 0.01 abcd	14.7 ± 2.5 abc
	WI3	4.2 ± 0.8 ef	3	0.11 ± 0.03 abcd	18.0 ± 5.0 abc
	WI4	2.2 ± 1.0 bc	1	0.08 ± 0.01 d	9.7 ± 3.7 c
Green Lake	None	0.0 ± 0.0 a	0	0.22 ± 0.04 ab	18.0 ± 5.9 c
	CA1	0.5 ± 0.8 bc	3	0.19 ± 0.06 abcd	30.7 ± 5.4 ab
	FL1	1.2 ± 0.4 cde	3	0.21 ± 0.01 abc	30.3 ± 2.5 ab
	FL2	1.2 ± 0.4 cde	3	0.21 ± 0.01 abc	31.0 ± 2.9 ab
	FL3	0.8 ± 0.4 bcd	3	0.20 ± 0.01 abcd	28.3 ± 2.4 abc
	FL4	1.8 ± 1.0 efg	3	0.18 ± 0.02 bcd	26.0 ± 9.1 abc
	FL5	1.5 ± 0.6 cde	3	0.16 ± 0.00 d	23.3 ± 5.8 bc
	NY10	2.3 ± 0.5 gh	3	0.23 ± 0.01 a	37.7 ± 2.6 a
	NY11	1.3 ± 0.5 def	1	0.20 ± 0.01 abcd	32.7 ± 3.9 ab
	NY12	1.8 ± 0.8 efg	3	0.21 ± 0.04 abc	35.0 ± 7.8 ab
	WI2	2.0 ± 0.6 fgh	3	0.18 ± 0.02 abcd	23.7 ± 4.7 bc
	WI3	2.7 ± 0.5 h	3	0.21 ± 0.01 abc	33.0 ± 2.9 ab
	WI4	0.2 ± 0.4 ab	2	0.17 ± 0.00 cd	27.3 ± 2.5 abc

[a] Average of six plants. Means followed by different letters differ significantly ($\alpha = 0.05$) according to Duncan's multiple-range test.
[b] Number of plants out of three from which CR bacteria were reisolated.
[c] Average of three plants. Means followed by different letters differ significantly ($\alpha = 0.05$) according to Duncan's multiple-range test.
[d] One plant with very slight yellowing.

lower concentrations of the inoculum. One uninoculated plant of the Salinas cultivar became contaminated, and one colony of the CR bacterium was isolated from that plant. The overall percentage of reisolation from infected plants was 90%. Shoot and root dry weights were significantly affected by cultivar and strain, but there was no significant interaction. The ranking according to Duncan's multiple-range test was not consistent with that for CR scores. The dry weights of the Green Lake cultivar were significantly higher than those of the Salinas cultivar ($F^{1,52} = 225$; $P < 0.01$).

Preliminary identification. All CR strains tested with Hucker Gram stain were gram negative. However, the KOH stringiness test was variable for the CR strains (Table 3). All New York strains, two Florida strains, and one Wisconsin strain showed a negative reaction (no stringiness), as did the California strain (CA1). The ability to form slimy strings in KOH was negatively correlated to the dryness of the strains on plates and the extent of flocculation in broth.

None of the CR strains were able to grow when held under anaerobic conditions for 3 weeks. However, the same cultures started to grow normally after removal from the anaerobiosis jar.

The oxidase test was positive for all strains (Table 3).

The catalase test was negative or very weakly positive when visually assessed on plates or in broth. Oxygen evolution as measured with an oxygen electrode was much slower for the CR strains than for the positive control (*P. fluorescens*), but the slopes of regression of percent oxygen on time were significantly higher for all CR strains than for *S. lactis* ($P < 0.01$). Thus, the catalase test was weakly positive for all CR strains (Table 3).

All the strains produced nitrite and ammonia from nitrate, but none produced nitrogen gas (Table 3).

The fatty acid profiles were very similar for all CR strains (Table 4). They consisted of several saturated and unsatura-

TABLE 3. Reaction of strains of the CR bacterium and control strains (*P. fluorescens*, *P. syringae*, *P. solanacearum*, *E. coli*, and *S. lactis*) to the KOH stringiness, oxidase, catalase, and nitrate reductase tests[a]

Strain	KOH test	Oxidase	Catalase[b]	Nitrate reduction		
				NO_2^-	NH_3	N_2
P. fluorescens	+	+	+	−	+	NT
P. syringae	NT	−	NT	NT	NT	NT
P. solanacearum	NT	NT	NT	(+)	+	+
E. coli	NT	−	+	+	+	−
S. lactis	NT	NT	−	NT	NT	NT
CA1	−	+	(+)	+	+	−
FL1	(+)	+	(+)	+	+	−
FL2	(+)	+	(+)	+	+	−
FL3	−	+	NT	+	+	−
FL4	(+)	+	(+)	+	+	−
FL5	−	+	NT	+	+	−
NY10	−	+	(+)	+	+	−
NY11	−	+	NT	+	+	−
NY12	−	+	(+)	+	+	−
WI2	−	+	(+)	+	+	−
WI3	(+)	+	NT	+	+	−
WI4	+	+	(+)	+	+	−

[a] +, Positive reaction; −, negative reaction; (+), weak positive reaction; NT, not tested.
[b] Oxygen production measured with an oxygen electrode.

TABLE 4. Fatty acid composition of whole cells of 12 strains of the CR bacterium as identified by gas chromatography-mass spectrophotometry[a]

Fatty acid	% Fatty acid in:											
	CA1	FL1	FL2	FL3	FL4	FL5	NY10	NY11	NY12	WI2	WI3	WI4
12:0–2-OH			Tr									
13:0–2-OH											Tr	
14:0–2-OH	6.1	8.4	8.5	6.9	7.7	5.9	5.7	9.1	4.1	7.5	5.6	6.0
15:0–2-OH											1.3	
12:0	Tr		Tr	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr
14:0	0.6	0.6	0.4	1.0	0.5	0.3	Tr	0.5	0.6	0.4	Tr	2.1
15:0	0.4	Tr					Tr		Tr		1.6	
16:0	13.2	12.7	12.9	15.1	9.7	11.1	14.1	11.1	9.8	10.0	7.4	18.0
16:1	10.8	15.4	10.4	13.9	9.6	12.1	12.1	11.8	12.2	10.6	7.6	7.1
17:0	0.4										1.3	
17:1	3.5	Tr					Tr	0.6	1.9		14.3	0.9
18:1	56.5	58.5	63.0	61.0	71.4	68.2	62.3	62.7	64.2	69.2	57.7	65.9
18:1–10 CH_3	5.9	4.2	4.2	Tr	0.5	2.4	5.3	3.7	6.4	2.1	2.7	Tr
19:0–cyclo	0.2	Tr	0.3	Tr	0.7	Tr	Tr	Tr	0.8	0.3	Tr	Tr

[a] Trace amounts not included in the calculation of the percentages.

ted straight-chain fatty acids, 2-hydroxy fatty acids, one cyclopropane fatty acid, and one methylated fatty acid. 3-Hydroxy fatty acids were not detected. The main straight-chain fatty acids had even numbers of carbon atoms (12:0, 14:0, 16:0, 16:1, and 18:1) with the exception of 17:1, which occurred in most strains from California, New York, and Wisconsin but not in those from Florida (except for a trace in FL1). CA1 and WI3 were the only strains with measurable amounts of 15:0 and 17:0. The main hydroxy fatty acid was 14:0–2-OH for all strains. All strains also had 10-methyl 18:1 fatty acid, and cyclopropane 19:0.

DISCUSSION

In a previous paper (23), we demonstrated that infectious CR of lettuce was caused by a gram-negative bacterium in California. In the current report, it was shown that similar bacteria resided in CR-prone soils from New York, Florida, and Wisconsin and that these bacteria induced CR on lettuce seedlings in the greenhouse. Although we have not tested the ability of these bacteria to cause CR under field conditions in the areas from which the soil originated (New York, Florida, and Wisconsin), it is likely that infectious CR is caused by bacteria similar to the California strain CA1.

All CR strains were pathogenic on the susceptible cultivar Salinas and to a lesser extent on the resistant cultivar Green Lake. The latter cultivar was developed in Wisconsin for resistance to CR at the time when the etiology of the disease was still unknown (21). Green Lake was later shown to be resistant to the California strain CA1 of the CR bacterium (5). Green Lake and breeding lines derived from it were also resistant to CR in Florida (12, 13). These observations lend weight to our proposal that CR in Wisconsin and Florida is caused by bacteria similar to those that cause CR in California.

Resistance to CR in Green Lake and other cultivars and breeding lines is conferred by a single recessive gene (5). When resistant and susceptible lettuce cultivars were inoculated with various strains of the CR bacterium, there was a statistically significant interaction between strains of the CR bacterium and cultivars in their effect on CR severity. However, disease severity on Green Lake was lower than that on Salinas for all strains, and the five most aggressive strains were the same strains for Salinas and Green Lake. We conclude that thus far no differential races of the CR bacterium have been detected.

All bacterial strains that induced CR were gram negative with Hucker Gram stain. The KOH stringiness test was variable. The New York strains and one Florida strain showed a negative reaction, as did the first California strain (CA1) (23). This indicated that these strains might be gram positive, but we demonstrated that strain CA1 contains lipopolysaccharide (23). Thus, the KOH stringiness test is not recommended for CR bacteria. Results with the traditional catalase test (visual observation of oxygen production) were also variable. This may be related to a relatively slow growth rate and possibly a slow metabolism. With the use of an oxygen electrode, we demonstrated that all strains of the CR bacterium were catalase positive. All CR strains reacted positively in the oxidase and nitrate reductase tests.

The fatty acid profiles were similar, consisting of several saturated and unsaturated straight-chain fatty acids, 2-hydroxy fatty acids, one cyclopropane fatty acid, and one methylated fatty acid. The most characteristic peak of the fatty acid profiles was that of a hydroxy fatty acid (14:0–2-OH). A large peak of this component is characteristic for *Pseudomonas paucimobilis* (19; M. Sasser, personal communication). However, the results of several other physiological and biochemical tests (unpublished data) indicate that the CR bacterium may not be the same as *P. paucimobilis*. No 3-OH fatty acids were detected. According to Oyaizu and Komagata (19), this is unusual for gram-negative bacteria. Another characteristic peak was that for 10-methyl 18:1 fatty acid, which has been reported for *Rhizobium* species (M. Sasser, personal communication). Since this fatty acid pattern is unique, it is likely that CR of lettuce is caused by strains of the same bacterium in Florida, New York, Wisconsin, and California.

ACKNOWLEDGMENTS

We thank Dan Jones of the Facility for Advanced Instrumentation, University of California at Davis, for the analysis of fatty acids. We are grateful to Myron Sasser of Microbial ID, Newark, Del., for performing some fatty acid analysis (to be published elsewhere) and for suggesting a potential relatedness between the CR bacterium and *P. paucimobilis*. We thank C. I. Kado for providing bacterial cultures.

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Newsletter Articles

Small Farms & Specialty Crops

Mark Gaskell, Ph.D., Farm Advisor

From our *Central Coast Agriculture Highlights* newsletter -- December 2000:

CORKY ROOT DISEASE OF LETTUCE

Figure 1. Resistant (Ultega) and susceptible (Parris Island) leaf lettuce varieties showing symptoms of corky root infestation.

Corky root disease is present in virtually every field that has a history of growing lettuce on the Central Coast. This disease has been present for a number of years and can cause significant crop loss at times. Corky root is a bacterial disease of lettuce caused by members of the Rhizomonas genus. Several species of Rhizomonas have been identified as causal agents of corky root in lettuce in a number of distinct conditions by Dr. Ariena H. C. van Bruggen of the Department of Plant Pathology at UC Davis.

Symptoms of corky root as seen above ground are poorly formed, discolored heads or tops with uneven, stunted growth depending upon the severity and timing of the infection.

Above ground, the plant develops poorly because the root system is unable to support the normal vegetative development. Severely infected plantings remain small and unmarketable (Fig. 1). In those cases where the infection occurs relatively late, the above ground symptoms may be minimal. At times, the plant may compensate for loss of a tap root with extended feeder roots and maintain sufficient root area to support the tops.

Below ground, the roots appear stunted, darkened in color with lesions and cracking (Fig. 2). In severe cases, roots are severely stunted and deformed with marked darkening, internal necrosis and, at times, with an army green coloration (Fig. 3.). The exterior tap root surface becomes pitted or corky.

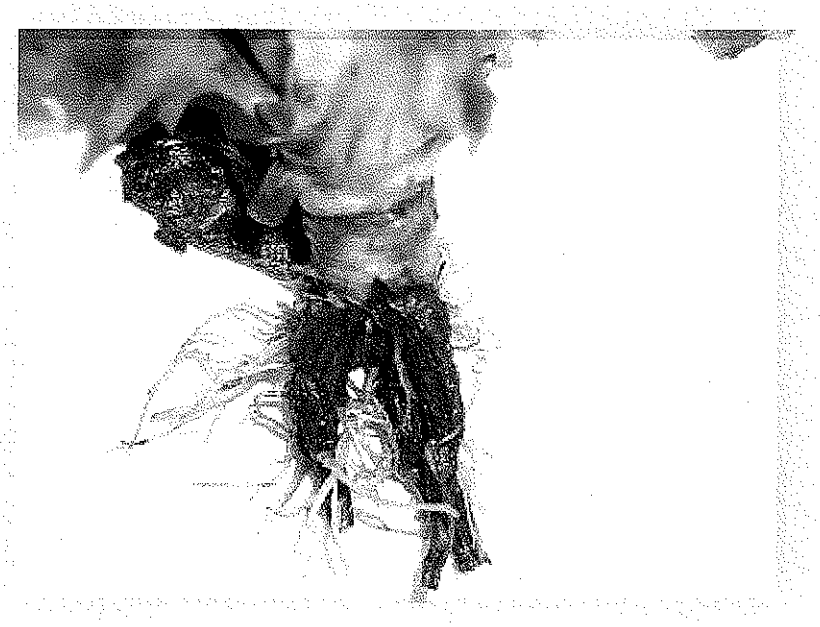

Figure 2.Stunting, deformation, pitting, corkiness and discoloration due to corky root disease.

Figure 3. Corky root susceptible Parris Island leaf lettuce (left), and resistant Ultega leaf lettuce from side-by-side rows.

Management of corky root disease in lettuce revolves around the use of resistant varieties where available and cultural practices. Some varieties are available commercially with resistance to corky root and the USDA lettuce breeding program in Salinas has an active effort ongoing to develop varieties with resistance to this disease. The Dutch varieties Ultega and Athena (Arden Zaden Seed Co.) showed marked resistance in corky root infested fields in Santa Barbara County in 2000. A number of other resistant varieties are also available.

Cultural practices for corky root management involve rotating lettuce crops, and soil and water management practices to improve aeration and drainage. Lettuce residue management is also important, and management of other residues may also contribute to the chances of infestation by corky root. The causal bacteria will survive for three years even in the absence of lettuce, but fields may be managed to minimize disease pressure. Excess moisture and poor soil aeration may contribute to corky root development, and soil ammonium ion levels may also be a factor. Compacted or poorly drained soils and extended or frequent irrigation also contribute to disease development.

It may be that a number of these factors interact in the field to create favorable conditions for disease development. Higher organic matter levels normally improve drainage and aeration in compacted soils. But the higher organic matter may affect the relationships between soil nitrogen dynamics and moisture to favor conditions for disease development. Organic growers or growers using reduced tillage to grow leafy crops should be especially aware of these factors. In these circumstances, high accumulated organic matter in surface soil may increase corky root incidence. Growers, working with fields with a history of corky root, should take care to rotate out of lettuce, allow time for residues to decompose completely,

carefully manage irrigation timing and duration, and use resistant varieties wherever possible. Anecdotal information suggests that the preceding crop may also affect corky root disease development. In side-by-side plantings in late summer 2000, leaf lettuce following cilantro showed little corky root, while leaf lettuce following spinach suffered nearly complete loss to corky root.

Dr. van Bruggen has evaluated several potential bacterial biocontrol agents for effectiveness in controlling corky root. In research, financed by the Iceberg Lettuce Advisory Board, Dr. van Bruggen reported significant differences in effectiveness among bacterial biocontrol strains, but no effect of application technique of these biocontrol agents. Although one or more of the bacterial strains showed a significant effect for controlling corky root disease, none of the biocontrol agents are available commercially at this time. For more information on corky root management in lettuce also see the UC Publication #3307, titled "Integrated Pest Management for Lettuce and Cole Crops," available from this office (805.934.6240) or online at http://www.ipm.ucdavis.edu/PMG/crops-agriculture.html

FRUIT AND VEGETABLE MARKET NEWS USER'S GUIDE

The USDA Agricultural Marketing Service has published a Fruit and Vegetable Market News User's Guide to assist anyone interested in using data collected through their extensive Market News Service network. Of particular interest to many are the price and volume reports for fruit, vegetables, and ornamentals for a number of shipping points and terminal markets in the US. Data is also available for several foreign markets for the past several seasons.

The guide describes the programs, reports, and services available through Market News Service and provides details of reading and interpreting these reports. The publication lists field offices that collect market information as well as those which also provide telephone-recorded information for daily updates. The guide provides addresses of field offices where hard copy reports may be ordered, or in some cases subscriptions are available for hard copy reports or daily reports via fax. Market reports are also available via the Internet at the AMS web site at:

http://www.ams.usda.gov/marketnews.htm

Some historical fruit, vegetable and ornamental price and volume data is available on the AMS web site. Additional historical records of the AMS data—as far back as 1990—are available through a web site maintained by the University of Florida at:

http://mis.ifas.ufl.edu/cgi-bin/barc/barc?a=d&k=fv

I have a few copies of the guide available through this office or copies may be ordered or downloaded from the USDA-AMS web site.

BACK TO SMALL FARMS PAGE

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Corky Root of Lettuce[1]

L. E. Datnoff and R. T. Nagata[2]

Corky root is a serious disease of lettuce. It has been reported in the states of California, Florida, New York and Wisconsin; and the countries of Canada, Great Britain, Greece, Italy, Netherlands and Spain. Yield losses for fresh and marketable head weights caused by corky root have been reported to range from 37 percent to 53 percent in Florida.

SYMPTOMS AND CAUSAL AGENT

Symptoms of corky root appear initially as yellow lesions, or bands on the tap and/or lateral root (Plate 1). As the disease progresses, the taproot becomes corked and brittle (Plate 2), and may exhibit vascular discoloration. Development of tap and lateral roots in infected plants are severely reduced or completely destroyed. Above ground, infected plants appear chlorotic and stunted. Uneven growth patterns are evident in the field. Heads are not formed at all or maybe unmarketable because of poor size (Plate 3).

Plate 1.

Plate 2.

Plate 3.

The etiology of this soilborne disease was unknown for many years. Its presence was attributed to numerous abiotic and biotic factors. However, in 1984, a slow-growing bacterium was isolated from diseased roots and, in 1988, was finally proven to be the causal agent of the disease. The name *Rhizomonas suberifaciens* has been proposed for this gram-negative bacterium.

EPIDEMIOLOGY

Little information is known about the biology and epidemiology of this pathogen such as the survival mechanisms and the influence of soil environment on disease development. This is due to the lack of a truly selective medium and the slow growth rate of this bacterium in culture. However, *R. suberifaciens* has been isolated from lettuce grown in fields recently brought into production after sugarcane. Similar observations have been made on lettuce grown in soil after pasture or forest. This organism also has been isolated from bean, melon, rye, and tomato, but only members of the Compositae closely related to lettuce (endive, common sowthistle, and prickly lettuce) are susceptible. Evidently, *R. suberifaciens* can survive in association with a number of crop and weed species, especially in root zones of these plants.

Soil type probably has little effect on disease development since corky root has been reported to occur on most soils used for growing vegetables. The pH of soils also seems to have little effect on corky root development, since in the laboratory *R. suberifaciens* has been reported to grow at pHs ranging from 5.7 to 8.2.

Severity of corky root will increase with applications of nitrogen fertilizer,

especially with side dressings of N such as urea. High soil moistures and temperatures also appear to favor disease development.

CONTROL

Fumigants such as dazomet, metam sodium and methyl bromide + chloropicrin are very effective for controlling corky root. However, the application of these materials probably are cost prohibitive on a commercial scale.

Host resistance also is very effective for managing this disease. Resistance is conferred by a single recessive gene. In Florida, several commercially resistant crisphead, romaine and buttercrisp lettuce types are available (Table 1).

Transplanting of corky root susceptible lettuce cultivars either 3 to 5 weeks old also is effective for managing this disease. This practice allows the use of susceptible types that otherwise would be lost.

Tables

Table 1.

Table 1. Commercial corky root resistant lettuce cultivars grown in Florida.

Crisphead	Romaine	Buttercrisp
Florical 50011	Augustus	Florida Buttercrisp
Greenlake	Floriglade	Florida 202
Montello	Tall Guzmaine	
Raleigh		
South Bay		

Footnotes

1. This document is Fact Sheet PP-50, a series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Publication date: June 1992.

2. L.E. Datnoff, associate professor, Plant Pathology, Everglades Research and Education Center (EREC), Belle Glade, Florida; R.T. Nagata, associate professor, Plant Breeding, EREC, Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville FL 32611.

The term "plates," where used in this document, refers to color

photographs that can be displayed on screen from CD-ROM. These photographs are not included in the printed document.

Copyright Information

HARRIS MORAN SEED COMPANY

PARRIS ISLAND COS

Romaine Lettuce

A superior romaine for your field

Parris Island is an excellent romaine that features the medium green colored leaves consumers demand. The plant is vigorous and uniform and produces abundant broad leaves. The leaves stand errect, sporting rounded tips, which are sparseley savoyed. The head is well folded and round on the top. This variety yields well and offers moderate bolting tolerance.

ADVANTAGES:

- ❑ Large upright plant
- ❑ Medium green
- ❑ Vigorous and uniform
- ❑ Moderate bolting tolerance

PARRIS ISLAND COS — **ITEM NUMBER: 14729**

Head Description:	Butt Appearance:	Leaf Type:
Medium-large, medium green	Large, flat	Romaine, blistered

PLANTING SLOTS

Target Markets:	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Coastal												
Desert												

Information given is an average of data gathered from our test locations. Your performance may vary depending on environmental and management conditions.
The complete Limitation of Warranty and Liability can be found in the Harris Moran Price List, at Harris Moran's website at www.harrismoran.com or by calling 1-800-320-4672.
For more information about our products and services visit our website at www.harrismoran.com.

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EXHIBIT E
STATEMENT OF THE BASIS OF OWNERSHIP

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF APPLICANT(S)

Paragon Seed, Inc.

2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER

PP 125

3. VARIETY NAME

Queen of Hearts

4. ADDRESS *(Street and No., or R.F.D. No., City, State, and ZIP, and Country)*

P.O. Box 1906
Salinas, California
93902-1906

5. TELEPHONE *(include area code)*

831-753-2100

6. FAX *(include area code)*

831-753-1470

7. PVPO NUMBER

200200029

8. Does the applicant own all rights to the variety? *Mark an "X" in appropriate block.* If no, please explain. [X] YES [ ] NO

9. Is the applicant (individual or company) a U.S. national or U.S. based company? [X] YES [ ] NO
If no, give name of country

10. Is the applicant the original owner? [X] YES [ ] NO *If no, please answer one of the following:*

a. If original rights to variety were owned by individual(s), is (are) the original owner(s) a U.S. national(s)?

[ ] YES [ ] NO If no, give name of country

b. If original rights to variety were owned by a company(ies), is(are) the original owner(s) a U.S. based company?

[ ] YES [ ] NO If no, give name of country

11. Additional explanation on ownership *(if needed, use reverse for extra space)*:

PLEASE NOTE:

Plant variety protection can be afforded only to owners (not licensees) who meet one of the following criteria:

1. If the rights to the variety are owned by the original breeder, that person must be a U.S. national, national of a UPOV member country, or national of a country which affords similar protection to nationals of the U.S. for the same genus and species.

2. If the rights to the variety are owned by the company which employed the original breeder(s), the company must be U.S. based, owned by nationals of a UPOV member country, or owned by nationals of a country which affords similar protection to nationals of the U.S. for the same genus and species.

3. If the applicant is an owner who is not the original owner, both the original owner and the applicant must meet one of the above criteria.

The original breeder/owner may be the individual or company who directed final breeding. See Section 41(a)(2) of the Plant Variety Protection Act for definition.